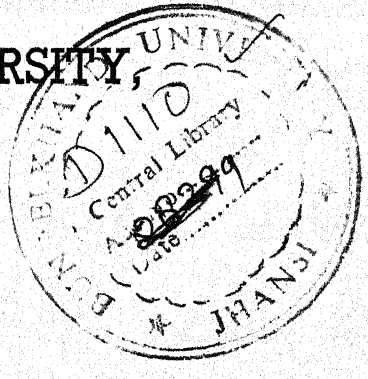
A SEROEPI DEMIOLOGY OF MALARIA IN A RURAL POPULATION OF PRIMARY HEALTH CENTRE, CHIRGAON, JHANSI (U.P.)

THESIS
DOCTOR OF MEDICINE

(SOCIAL AND PREVENTIVE MEDICINE)

BUNDELKHAND UNIVERSITY,
JHANSI (U. P.)



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Kamal Kishore Re

C E R T I F I C A T E

This is to certify that the present work entitled "A SERO-EPIDEMIOLOGY OF MALARIA IN RURAL POPULATION OF PRIMARY HEALTH CENTRE, CHIRGAON, JHANSI (U.P.)" has been carried out by Dr. KAMAL KISHORE REMY himself in this department.

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C E R T I F I C A T E

This is to certify that the present work entitled "A SERO-EPIDEMIOLOGY OF MALARIA IN A RURAL POPULATION OF PRIMARY HEALTH CENTRE, CHIRGAON, JHANSI (U.P.)", has been carried out by Dr. KAMAL KISHORE REMY, under our constant supervision and guidance. The observations were checked and verified by us from time to time.

This thesis fulfils the basic ordinance governing the submission of thesis for M.D., laid down by Bundelkhand University.

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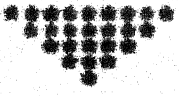

INTRODUCTION

INTRODUCTION

Malaria has reappeared during the last decade throughout the tropical and subtropical regions of the world. In the world today, there have been radical changes in socio-economic conditions and some consequences of development have led to changes in habitats and ecosystems of malaria vectors and parasites. Developments in agriculture such as intensification and reorientation, green revolution, irrigation, deforestation, development in the industry and other technical interventions; high mobility of population as well as increased labour force movement, resettlement and rehabilitation, increased tourism, have all introduced new aspects in the epidemiology of malaria. The development of drug resistance by the parasite and insecticide resistance by the vectors are the prime causes for the reappearance of malaria in the areas where it was practically controlled previously. Failure of operational and surveillance measures were also equally responsible for the resurgence of malaria.

The revised global plan for achieving the containment of the virulent malaria with the ultimate aim of eradication of the disease envisages, among many

other aspects, a proper estimation of the extent to which population in endemic areas has been exposed to malaria.

It is observed that malaria had, in fact, been erradicated in over 20 countries, freeing a population of nearly 800 million from risk of the disease and transmission of the disease had also been gradually reduced in areas inhabitated by 775 million people.

It has also been observed that modification of clinical illness occurs in endemic areas, due to development of immunity. In such areas parasitaemia may be seen without clinical illness and vice versa and also the individuals may be missed by screening of fever cases only. Parasitaemia in malaria is intermittent and its absence on single slide examination does not exclude the diagnosis of malaria. This limits the usefulness of slide examination for malaria detection epidemiologically. Secondly, slide surveys of the population are cumbersome and do not yield results commensurate with the work involved. Another indicator of prevalence of malaria has been the spleen rate. This is a good index for giving on the spot diagnosis, but it does not hold good in areas with low endemicity. Besides malaria, there are other causes of splenomegaly and it is not enlarged in all patients of malaria, hence there is

a need for a newer method, which is better indicator of endemicity and transmission of malaria and involves less expense, time and labour.

Serological tests for malaria have been reported to be useful tool for studying malaria endemicity rates, patterns of malaria transmission and to detect foci of malaria in epidemiological survey (Kagar, 1972).

Of large number of serological tests available, ELISA & IIF test have been found to be simple, reliable, reproducible sensitive, specific and large number of samples can be economically processed (Voller et al, 1980). Many studies have been conducted abroad but it needs evaluation in Indian conditions. Another aspect of serology of malaria which needs evaluation, is the use of serology for the diagnosis of individual patient or community diagnosis. No single test has been found useful in this respect but use of multiple tests needs evaluation. Keeping these facts in mind, the present study has been designed with the following aims and objectives :

1. To assess the prevalence of antibody titre in random population.

2. To find out correlation between sero-positivity and slide positivity.

3. To evaluate application of serology to study the epidemiology of malaria by correlating it with various bio-social characteristics of the population.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

The word malaria is derived from an Italian word, Mala-aria which stands for bad airs. In 1740, Horace Walpole, for the first time used the word Malaria in English.

The term Malaria is applied to a group of diseases caused by infection with specific sporozoon parasites of genus plasmodium and transmitted to man by various species of Anopheline mosquito transmit the disease which is clinically characterised by episodes of chills and fever with period of latency, enlargement of spleen and secondary anaemia (Park & Park, 1989).

Malaria is caused by four distinct species of plasmodium, viz. P. vivax, P. falciparum, P. malariae and P. ovale. Agent requires two hosts for its propagation and completion of life cycle. The disease affects individuals of all ages and both sexes especially of low socio-economic status, living in ill-ventilated, ill-lighted and unhygienic houses surrounded by various types of water collections. The female anopheline mosquito, the definitive host, requires 20 - 30°C temperature and high humidity for active life. Such conditions prevail during rainy season, when the disease flares up.

Though the disease is wide-spread all over the tropical belt, it presents with varying degree of endemicity, the seasonal variation host and vector factors responsible for the disease. There is need for an economical, simple, safe, reliable, sensitive, specific method for assessing the extent of the problem. Three methods viz. spleen rate, parasite rate and serology are in use. Ideally, a method should identify the infecting species differentiate between present and past infections and indicate the immune status of the host. A test that fulfilled these goals would be both sensitive and diagnostic method and also a valuable tool for the study of epidemiology of malaria. In view of this, serology seems promising in areas of high incidence malaria; the age groups above 4 years acquire immunity induced by repeated infections, resulting more number of unrecognized cases of malaria.

Although no method has fulfilled these criteria; yet some serological methods have proved to be a valuable tool to assess more accurate appreciation of the prevalence of malaria, especially in circumstances where the use of anti-malarials, invalidates the classical parameters of malaria endemicity such as the prevalence of spleen enlargement and parasitaemia (W.H.O., 1975).

1. <u>History of Disease</u> :

Ancient history during vedic times, malaria is reported as "King of diseases" and was often attributed

due to anger of lord Shiva in medical literature (Charaka Samhita & Susrute Samhita, 600 B.C.).

The Chinese, centuries before the Christian era, differentiated tertian from quartan fevers and recognised the enlargement of spleen in malaria.

The year 1880 is important in the history of Malaria as it marked the discovery of malaria parasite in fresh human blood by Laveran and then Romanowski gave to the world an original technique of staining blood smears.

Ronald Ross (1898) discovered the life cycle of Malaria parasite in the invertebrate host (The mosquito). The introduction of plasmochin (now called Pamaquin, 1924), a synthetic anti-malarial in its chemotherapy followed by Atebrine (1930) now called mepacrine, chloroquin (1934) and Paludrine (Proguanil, 1945) were outstanding discoveries in the field of malariology (Central Health Education Bureau, C.G.H.E. Ministry of Health, Govt. of India).

Firstly, in the control methods re-discovery of Pyrethrin (1936) a contact insecticide marked the beginning of malaria control measures and later, followed by residual synthetic insecticides like D.D.T., B.H.C., Dieldrin and other organophosphorous compounds etc. and proved practicable, economicelly feasible in rural areas, towns and cities (Central Health Education Bureau, 1959).

World Health Organization from its inception has recognised malaria as a major health problem, out of six tropical diseases, malaria was the target of W.H.O. special programme for research and training in tropical diseases to develop new tools and strengthen research institutions and training workers in the countries affected (Park & Park, 1989).

2. Epidemiological features of Contemporary Malaria :

Malaria is widely prevalent throughout the world with a high prevalence in Asia and Africa. It was established that the population at risk of malaria (excluding China), was about 1729.17 million in 106 countries of the world. Out of which, the eight countries of South-East Asia contributed for 918.72 million people (W.H.O., 1982).

The resurgence of malaria had occurred throughout the world, with a peak in 1976 followed by decrease in the number of cases so that by 1982 the number was almost similar to that reported in 1974 (TRS - 735). By end of 1974, there were 1,136 million people at the risk to suffer from malaria (Srivastava et al. 1975) in India.

2.1 Demographic explosion :-

The average growth of more than 2% population was significantly increased among economically backward

classes, inhabiting areas with difficult accessibility on the periphery (Ray, 1979; Kondra Shin, 1983).

2.2 Intensification and Re-orientation of agriculture :

About 70 to 80 percent of population being engaged in farming in the country. It was estimated that there were about 50 million people engaged in seasonal agricultural activities and moving for purposes of harvesting throughout the country (Kondrashin, 1983).

2.3 Industrial Development :

Production of steel, electronic goods, heavy machines, fertilizers, exploration of oils, bamboo cutting, land clearance, gem-mining, coal fields, ore mines, thermal power stations, roads, lime store, dolomity, quarts, aluminium, copper etc. The mineral wealth of the country lies in areas of difficult terrain, mostly in hills, which are hard core areas (Ray, 1979).

2.4 Urbanisation :

It may be seen that both natural (high temperature and humidity vast areas of vector breeding places, prolonged transmission season etc.) and socio-economic factors (low sanitary standards, intensive population movement, demographic explosion etc.) create extremely favourable conditions for transmission of vector borne diseases in urban areas.

2.5 Improvement in transport facilities :

To support mining, forestry and industrial projects, the road communication has increased tremendously. It is estimated that around 50% of labour is imparted from other places in the country consisting of landless workers. They carry infection from hard core areas to urban as well as rural areas.

2.6 Deforestation :

In forest based industries, shifting agriculture is still in use, resulting in massive deforestation which in turn results in degradation of land, soil erosion and sedimentation of lakes, river and reservoirs with increase in the density of mosquito (Kondrashin, 1987).

2.7 Irrigation :

On the whole, it appears that in India, canal irrigation leads to a marked rise in the ground water table and any disturbance in natural drainage contours sometimes obstructs the natural drainage flow of rain water in the area.such changes create favourable malariogenic condition with high transmission potential.

2.8 Unemployment & under-employment :

The most important of Indian economy, the unemployment problem is essentially rural in character

and has grown vastly in magnitude the absolute number of unemployed on a typical day about 21 million constituting 8.2 percent of the labour force (Agarwal, 1985). There could be an epidemic out-break of malaria in the labour camps and increased vulnerability of malaria in the labour camps and in the originating areas on account of the return of labourers.

The labour force are being made at a fast rate on account of the rapidly growing population. Thus, while new productive jobs are on the increase, because of the low rate of increase, the absolute number of unemployed persons is rising from year to year (Agarwal, 1985).

2.9 Tourism and Pilgrimage :

The celebration of religious festivals like Kumbha Mela results in the congregation of many hundreds of thousands even millions of people at a time. The results of random study conducted on a group of pilgrims for several states of India have shown that the parasite rate among them was about 2% and all cases were due to P. falciparum, a symptomatic gametocite carriers (Raj Gopalan et al, 1986).

Malaria is not only a rural problem but is also important in urban areas.

3. Age/Sex/Occupation related Malaria :

3.1 Age related malaria :

The prevalence of malaria among different age groups is subject to wide variations. In a primary health centre Nainital district of Uttar Pradesh, Choudhary et al (1984) carried a study and classified the population on the basis of clinical history of malaria observing that all age groups were affected by disease but that there was a progressive increase of malaria attacks from infants to 16 - 25 years of age, when the rates reached the maximum level the NICD has conducted serological studies in hypo-endemic malarious areas of the country during the 1970s and it was observed that the population below 5 years of age had hardly any malaria experience. It was only higher age groups who showed high titres. Kumar et al (1986) in a study around Delhi i.e. Sonepat; Gurgaon and Ganiabad, revealed ELISA antibodies showed a very definite trend towards age related increase in the titre, employing P. falciparum antigen, gave desired results. Both the IHA & ELISA mean titres correlate well with S.P.R. in the non-transmission period such a correlation was lacking in other period, the expected age related increase in antibody titres was evident with ELISA and not IHA.

Kumar et al (1987) reported that the immunological profile of a population was the sum total of previous individual experiences. The response of the individual

to these experiences was affected by age, immunological competence, cumulative exposure to malaria antigen and kind and amount of specific therapy used, and also reported age related increase in the number of individuals with positive peripheral blood smears increase upto the age of 4 years and then there was a sharp decline. The infection rate was found to be 26.9 in the age group 1-5 years. The infection rate was calculated for each village individually which ranged from 1.7 to 63.2. There was a good correlation in the age group 1-4 years between the infection rates of each village and total number of malaria cases as percentage of entire population of each village.

3.2 Sex related malaria :

Sex differences between the general distribution of these in Indian population and in the percentage of malaria cases reported for each category probably are influenced by local socio-economic status, ethnic groups, the attitude of parents especially mothers towards males and female children treatment for malaria, ignorance about the availability of free services in the village. In Uttar Pradesh state, Chowdhary et al (1984) observed that both sexes were affected by the disease but incidence among males was almost twice as high as compared with that among females.

3.3 Occupation related malaria :

Different occupational categories of labour force were identified as being at increased risk to acquire malaria, including its resistant forms and disseminating the disease. The overwhelming majority of total malaria cases annually occurs among various categories of agricultural labour (Pattanayak, 1981). The rest of the cases occur in urban and other areas of the country (Sharma, 1984; Kondrashin and Dixit, 1985). The risk to acquire malaria is high among mobile workers and among those exposed to mosquito bites in the open air on account of their occupational requirements (Kondrashin, 1986). The higher S.P.R. was reported in labourers engaged in bamboo cutting, tea plantations, coal fields, coconut plantation, fishermen (Panicker et al. 1984; Panicker & Rajgopalan, 1986).

4. Malaria in India :

Malaria is one of the great scourages afflicted humanity. Even early in this century there was no aspect of life in our country which was not affected either directly or indirectly by Malaria (Sinton, 1936).

At the time of independence, malaria was regarded as a major public health problem. The annual incidence of malaria was 75 million cases with 8 lakh deaths directly due to malaria (Park & Park, 1989). In post independent era,

Govt. of India realising gravity of problem, started National Malaria Control Programme in 1953, which upgraded to National Malaria Eradication Programme in 1958 due to fear that vector may develop resistence to insecticides (Malaria in India, 1958).

During the 'phase of attack' positive incidence of malaria dropped down from 75 million cases to 2 million cases in 1958 and the proportional case rate fell from 10.8 in 1953 to 3.2 in 1958 (Park & Park, 1989). Progress in malaria control was also seen in neighbouring countries of South East Asia Region of W.H.O. Five had progressed beyond the attack phase except Nepal, 19 - 77% of population of other five countries had reached the 'consolidation phase'.

In India itself, 99% population was covered by attacked phase and 1% by consolidation phase in 1961. By 1966, 14% population was in attack phase, 34% in consolidation phase and 52% was under maintenance phase.

The success of the malaria eradication programme was due to normal sensitivity of parasite to chloroquin, and sensitivity of anopheline vectors to D.D.T. However, programme suffered a set back after 1965 in the form of small and large focal outbreak of malaria in different states of the country. Disease showed an upsurge trend year after year till the condition started worsening from 1975. There were 13,58,753 cases of malaria in 1971 which

increased to 53,10,790 in 1975. Considering the gravity of the situation, a modified plan of operation was implemented in 1978.

There were 64,67,215 cases of malaria in 1976 which decreased to 17,65,631 in 1986. The status of malaria in the country in last 11 years is shown in table 2.1.

Table 2.1

Year	Total malaria cases	P.falciparum cases	Annual parasite incidence	S.P.R.
1976	64,67,215	7,53,713	11.26	11.553
1977	47,40,900	4,59,867	8.07	8.316
1978	41,44,385	5,48,567	6.80	6.855
1979	30,64,697	5,58,423	4.90	4.990
1980	28,98,140	5,88,011	4.51	4.315
1981	27,01,141	5,89,591	4.11	3.982
1982	21,82,302	5,51,057	3.22	3.356
1983	20,18,605	6,00,694	2.93	3.140
1984	21,84,446	5,86,691	3.08	3.290
1985	18,64,380	5,45,005	2.57	2.740
1986	17,65,631	6,21,235	2.40	2.660

Source : W.H.O./SEARO, 1987.

Malaria is not only a rural problem but is also important in urban areas. Pattanayak et al (1981) had shown that Madras city contributed 40 - 50% of total malaria cases of the state. The number of malaria positive cases in ten major cities in India between 1978 to 1985 are shown in Table 2.2.

Table 2.2

Malaria cases in ten major cities in India (1978, 1980-1985).

Cities	Pop. in millions	Malaria cases						
		1978	1980	1981	1982	1983	1984	1985
Ahmedabad	2.52	26705	26664	20703	12086	18329	23186	16113
Bombay	8.23	2635	1608	309	N.A.	3700	2610	1371
Baroda	0.74	29866	12161	13648	8743	8378	5769	6562
Bangalore	2.91	952	322	216	101	59	34	29
Bhopal	0.67	2656	2893	N.A.	1103	2055	2740	2746
Chandigarh	0.42	34748	38278	31209	25945	28835	24035	36545
Calcutta	9.17	1244	3246	5527	5304	19370	26056	21303
Delhi	5.71	332683	69277	62415	46530	4107	38108	28577
Hyderabad	2.53	2559	1242	2494	4337	2480	3346	4096
Madras	4.28	24953	36193	43981	44981	44817	48523	51376

Source : NMEP, Delhi 1987, based on 1981 census.

Table 2.2 shows that the cities of Delhi, Madras, Calcutta, Ahmedabad and Chandigarh are the main contributors to the problem of urban malaria in India at present. This was

particularly so in the year 1978, when the number of malaria cases in only ten major cities of the country accounted for more than 11 percent of total malaria cases in the country. This problem have infact been on the increase since the year 1982 onwards.

In Lakshadweep Island, Ray et al (1978) observed the A.P.I. of 102, 445.2, 20.2 from Miruccy, Bitra and Chetlet Islands respectively. In all above studies parasite was sensitive to chloroquin.

Shanmugham et al (1978) from Tamil Nadu also reported 6656 cases of P.Falciparum and treated them with 1200 mg and 600 mg chloroquin base for adults and children respectively. Monthly follow-up of these patients was done after the treatment. Only one patient showed parasite in peripheral blood. Soon it was observed that P.Falciparum in certain parts of India had developed chloroquin and multiple drug resistence. The chloroquine resistence against P.Falciparum was first detected in Assam (Sehgal et al 1973) subsequently chloroquine resistance was detected in Arunachal Pradesh, Mizoram, Meghalaya and Nagaland (Pattanayak et al 1979; Chakraborty et al 1979 and Das et al 1979). The resistence was also found in Maharashtra, Orissa, Uttar Pradesh and Madhya Pradesh (De, et al. 1979; Guha et al. 1979 and Dwivedi et al. 1981; Baveja et al. 1985). These strains are spreading in virulent form causing high degree of morbidity and mortality in children. Krotoski(1981)

observed that some South East Asian strains of P. vivax were also resistant to standard regimens of Primaquin.

Malaria control in rural areas was carried out by spraying residual insecticides such as DDT, HCH or malathion. In areas with DDT resistant vectors, HCH was sprayed. At present about 210 million population is under DDT spray and about 100 million under HCH spray.

Malathion resistance in A. culicifacies developed in areas of Orissa and Andhra Pradesh, where this insecticide was never used in public health, this development of resistance in A. culicifacies was the result of the use of organo phosphate compounds in agriculture (Nagpal, 1986; Sharma, 1987 b). At Shahjahanpur, the bio-environmental control of malaria strategy was implemented in 1986, results so far achieved have shown major reduction in vector densities and SPR was reduced from 80 - 90% to 20 - 30% (Sharma, 1987 b).

5. Malaria in Uttar Pradesh :

Utter Pradesh is the largest state of India with a population of 110.9 million (Census, 1981).

Malaria eradication programme was launched in the state during 1958-59. By 1959-60, sixty seven eradication programme units were established in the state covering 67 million population of the state. Barring the population

living in areas above 5000 ft, the entire state was covered under the programme (State Health Education Bureau, U.P., 1987).

The entire state of Uttar Pradesh remained in attack phase till 1961-62. The Units which had achieved the desired criteria were recommended for entry into another phase by the independent Appraisal Teams, started entering into consolidation phase' from 1962-63 and into maintenance phase from 1965-66. By 1969-70, out of 67 units, 51.27% units entered into maintenance phase (State Health Education Bureau, U.P., 1987).

During the period 1951-1964 the incidence of malaria was brought down to a very low level and the state was almost free from malaria. The programme suffered set backs in some units from 1965, and the disease spreaded and showed an upward trend year after year from 1965 to 1977. 4,33,944 cases were seen in 1977. The year-wise data from 1970 to 1977 is as follows (Table 2.3).

Table 2.3

Year	No.of malaria cases detected
1970	7,802
1971	9,798
1972	17,499
1973	54,145
1974	1,90,755
1975	3,81,750
1976	3,87,728
1977	4,33,944

There has been a considerable improvement in epidemiological situation since 1977. But during 1979 the incidence of malaria cases was on the increase till 1984. However, during 1985 the incidence of malaria has declined by 11% as compared to the year 1984. The incidence of malaria cases was further declined 66.17% in the year 1987 as compared to 1985 (Table 3) (State Health Education Bureau, U.P., 1987).

Table 2.4

Epidemiological data from the years 1977 onwards is as follows :

Years	Positives	A.P.I.	S.P.R.
1977	4,33,944	4.4	5.8
1978	3,60,059	3.3	4.1
1979	1,49,919	1.5	1.9
1980	1,82,308	1.7	1.9
1981	1,75,930	1.6	1.9
1982	1,70,233	1.6	2.2
1983	2,85,618	2.6	3.5
1984	4,19,708	3.6	4.5
1985	3,73,006	3.1	4.2
1986	2,28,244	1.09	2.9
1987	1,26,181	1.04	1.58

API : Annual parasite incidence, SPR : Slide positivity rate.
Source : Deptt. of Malariology, Govt. of Uttar Pradesh.

6. Malaria in Jhansi :

It has the population of 11,37,031 and having 836 villages (Census, 1981). This district is having hot and dry climate. Srivastava et al (1975) in a study had showed the mean monthly maximum and minimum temperature range between 24.1°C to 42.6°C and 9.2°C to 29.3°C respectively. The mean monthly relatively humidity ranges between 26 to 84% at 0830 hrs. and 15 to 76% at 1730 hrs. Mean monthly rainfall ranges from 2.7 mm in the month of April and 339.1 mm. in the month of August. There were 104 surveillance units with a total of 1312430 surveillance population in 1973 (Srivastava et al. 1975). Data presented in the table 2.5 reveals that the incidence of malaria cases showed a rising trend from 1980 to 1984, thereafter they started declining. It was observed that there was a fall of about 92% in the number of positive cases in the year 1987 in comparison to 1984.

Table 2.5

Year	Incidence of malaria in Jhansi	A.P.I.
1980	1434	7.6
1981	2155	6.7
1982	1869	7.0
1983	2093	7.0
1984	7594	-
1985	7591	5.6
1986	5444	5.1
1987	3991	3.7

Source : District Malaria Office, Jhansi.

In the year 1987, status of malaria at Primary Health Centres of Jhansi is shown in table 2.6.

Table 2.6

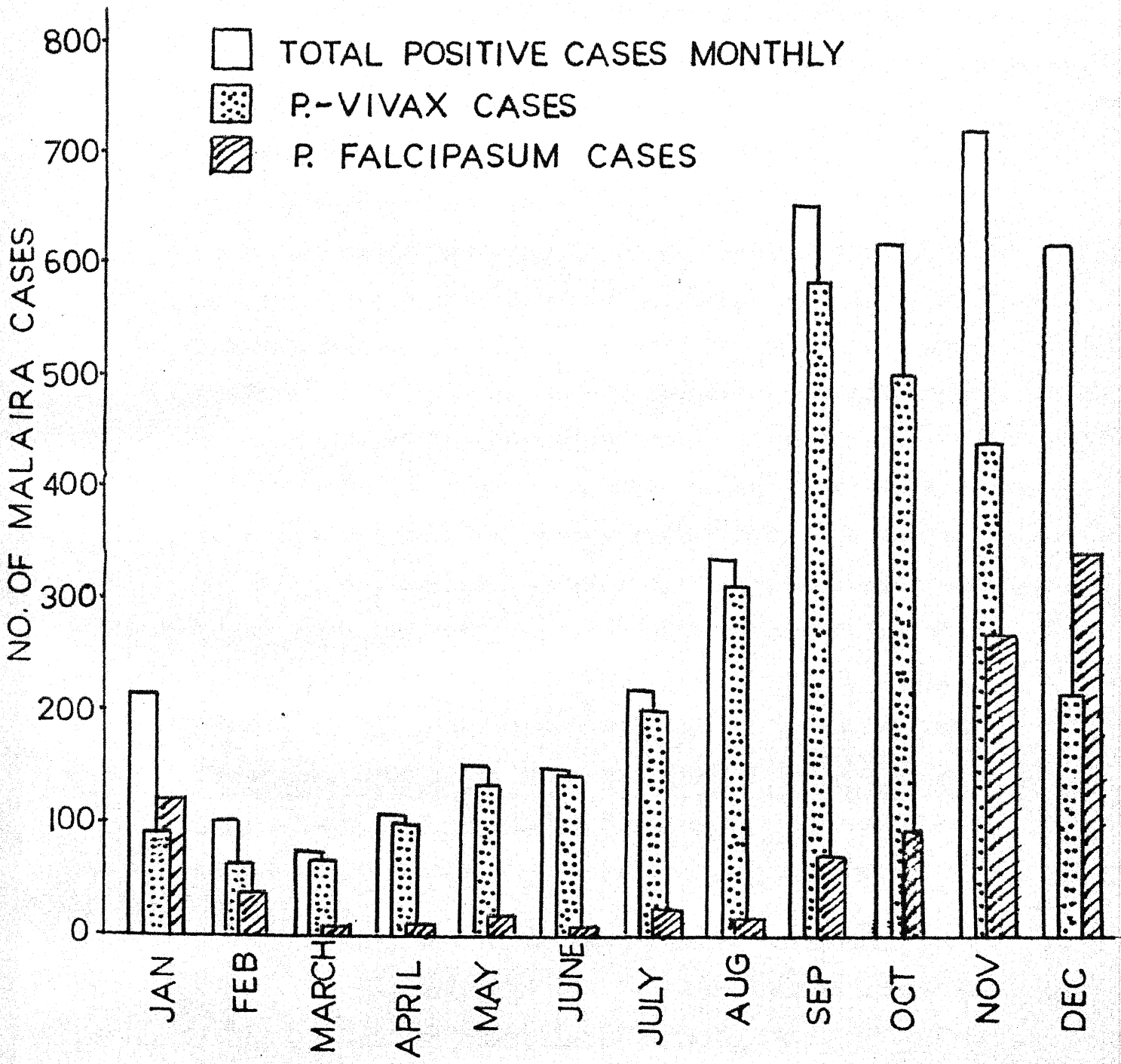
Name of P.H.C.	No. of malaria cases detected	A.P.I.	S.P.R.
Rakina	555	4.8	7.4
Mauranipur	682	4.7	7.5
Gursarai	76	0.64	0.75
Bamore	159	1.4	2.5
Baragaon	371	3.6	9.6
Chirgaon	275	2.6	2.8
Moth	650	4.7	6.8

Source : District Malaria Office, Jhansi.

In a study, Srivastava et al (1975) and Verma et al (1975) had found that P. vivax infection was responsible for 82.25% morbidity and A. culicifacies and A. fluviatilis are two known vector of malaria in Jhansi, with former playing the major role in the transmission of the disease. They have also observed favourable transmission season for the species P. vivax and P.falciparum, from August to October as peak month and minimum load of infection during January and December. The monthly malaria positive cases in Jhansi depicted in figure 2.1.

FIG - 2.1

BAR DIAGRAM SHOWING MONTHLY MALAIRA POSITIVE CASES DURING 1987 IN JHANSI DISTRICT



7. Spleen rate :

The spleen rate is the proportion of children (aged 2 - 10 years) in a community who have large spleen. The spleen rate includes past as well as present malaria infection and it is frequently more reliable in practice than examination of blood films (Mac Donald, 1957). Hackett's method is used for classification of enlarged spleen. Spleen size is not considered for determining the spleen rate itself, but it is used to calculate average enlargement of spleen, another indicator of endemicity. In the holo- or hyper-endemic areas of malaria, the A.E.S. in age group 2 - 9 years is high and in the epidemic areas is low in the same age group. After interruption of malaria transmission in highly endemic areas, spleen rate remain the same for months or diminish insignificantly but on the other hand the A.E.S. remarkably decreases, therefore, is a more sensitive tool in evaluation than the spleen rate alone (Marshal, 1986). The spleen rate taken among the 2 - 9 years age group and determination of A.E.S. are easier to obtain than parasite rate.

Spleen rate is a good index for diagnosis of malaria during epidemics and in hyper-endemic areas as it gives on the spot epidemiological situation, the degree of immunity, previous history and future prospects of disease. No other disease is known to cause such a high

spleen rate except Kala Azar. But then, all patients with splenomegaly do not have malaria and all patients of malaria do not have splenomegaly.

8. Parasite Rate :

The parasite rate is the proportion of a population in which malaria parasites are found. Infant parasite rate is defined as the percentage of infants below the age of one year showing malaria parasites in their blood. However, it is an important procedure in both individual diagnosis and epidemiology and has been main stay of parasitic detection in infected mosquitoes and in man, till relatively simple diagnostic tests are developed (W.H.O., 1987).

Blood examination for malaria parasites provide a seasonably adequate measure of the point prevalence of the infection. For community application, it requires large number of field workers as well as technicians. Non-fever cases are not studied, so sub-clinical infection without fever remains undetected. In a study in meso-endemic area, Upreti et al (1982) obtained 6.8% slide positivity rate in a febrile healthy children of 2-9 years, although the positivity was higher (45.5%) in febrile ones. Slide examination can only indicate the presence and absence of patient parasitaemia at the time of examination; it does not indicate the individuals malaria experience (Kagan, 1972).

The results of spleen surveys are compared with other epidemiological methods in following table.

Comparison of methods of epidemiological investigations in malaria.

Sl. No.	Author		Spleen rate %	Parasite rate %	Sero-positivity %	Comment
1.	Sharma et al (1985) (2-9 years)		30.65	14.6	Not done	Spleen was found in many parasitologically negative cases.
2.	Upreti et al (1982) (2-9 years)		29.4	6.8	"	Splenomegaly detected in many parasitologically negative cases.
3.	Mamtani et al (1979)		3.2	7.8	36.0	Of 36 persons with enlarged spleen, only 4 had parasitaemia.
4.	Van Der Kaay et al (1973)					Results of sero-positivity and parasite rate provide a reasonably adequate measure of point prevalence and degree of malaria endemicity.
	2-9 years		90.0	75.0	-	
	7/ 15 yrs.		70.0	37.0		
5.	Kaay et al (1973)		90.0	53.0	78.8	There was no correlation between splenomegaly and seropositivity.
6.	Collins & William et al (1972)					
	1-5 years			91.0	78.4	Sera taken at different times of the year gave differing picture of endemicity.
	Area (a) 7 15 years		Not done	49.0	92.8	
	Area (b) 1-5 years		"	78.0	94.0	
	7 15 years		"	35.6	99.8	
7.	Denowitz et al (1965) (2-9 years)		87.0	72.2	81.7	--
8.	Voller et al (1959)					
	2-9 years	(A)	46.9	77.9	90.0	Spleen rate was much lower than parasite rate and seropositivity.
		(B)	50.0	81.0	80.0	
9.	Patel et al (1961)	(A)	7.5	9.3	Not done	In low endemic areas spleen rate is not useful.
		(B)	0.0	0.14	"	
		(C)	0.0	1.04	"	

The absence of patent parasitaemia can be misleading since patency is influenced by immune status, and use of anti-malarial drugs. Besides parasites may not be present in peripheral blood continuously during cycle, especially in falciparum malaria where only ring stages develop in peripheral blood and other stages develop in visual capillaries. Furthermore, unless several blood surveys are carried out at different time of the year, and are combined with spleen rates, it is not possible to predict with any degree of certainty the amount and intensity of perennial malaria endemicity in a given area (Voller and O'Neill, 1971).

9. Serological method :

Serological tests such as indirect fluorescent antibody (IFA) test and to a lesser extent indirect (passive) haem-agglutination tests, enzyme linked immuno-sorbent assay (ELISA), and gel precipitation tests also have a diagnostic role in epidemiological studies. In the past, these tests had suffered disadvantages due to non-availability of pure forms of antigens or antibodies.

The recent isolation, however, of purified antigens from all stages of parasite and the development of specific monoclonal antibodies have resulted in the development of more specific reagents and a new generation of tests. These, alongwith the recently identified

parasite-specific DNA probes, have increased the prospects of applying to epidemiological studies new and improved diagnostic tests, capable of automation (W.H.O., 1987).

A great variety of antibodies are produced during the course of malaria infection. These may be detected by precipitation of soluble antigens, fluorescence, agglutination opsonization of parasitised cells, and blocking of merozoite penetration (Playfair, 1978). Opsonizing antibodies (Rogers, 1974), merozoite blocking antibodies (Mitchell et al, 1975) and delayed hypersensitivity (Phillips et al, 1978) provide protection from reinfection but are slow to develop. The precipitin, fluorescence and agglutinin antibodies rise early during infection and persist for long periods. The role of serological procedure like indirect fluorescent antibody test is very promising in the epidemiologic interpretation of data in malaria (Draper et al, 1972; 1972 b; Voller, 1971). The period prevalence of malaria in the community as seen by age related antibody profile has been shown to be a more sensitive tool for surveillance as compared to parasite index which provides point prevalence data (Draper et al, 1972 a; Bruce Chewatt et al, 1975; Lobel et al, 1976; Kumar et al, 1986, 1987). IIF provides period prevalence data which was more informative as compared to point prevalence data given by parasite rates (Collins et al, 1967; Mc Farlane et al, 1970; Meuwissen,

1974; Spencer, 1979). Serologic data seem to be more consistent with parasite indices in stable transmission area (Draper et al. 1972 b). Kumar et al (1987) reported the use of serological procedures in the measurement of malaria in a community and shown it to be more reliable than conventional methods like the parasite index. The infection rates of malaria in the community as calculated from the serological data confirm the reliability of serology in the measurement of malaria.

Sero-positivity correlates well with parasitaemia more so during the transmission season. The amount of antibodies were higher in the non-transmission season as observed by Kumar et al (1986). Lower antibody titres during the peak transmission could be due to absorption of antibodies by the parasite in the blood at a rate faster than they are produced.

Repeated exposure in endemic areas should result in increase antibody levels which would be reflected in an age related increase (Collins et al, 1967; Draper et al. 1972 b). Therefore, particularly in higher age group. there may not be parasites in blood due to immunity, thus peripheral smear examination has certain limitation. In view of this, serology seems promising in the areas of high incidence of malaria; the age group above 4 years acquire immunity induced by repeated infections, resulting

more number of unrecognised cases, and therefore, no correlation with overall incidence but within the age groups of 1 - 4 years of age as expected, there was good correlation of antibody status with incidence of malaria. However, since serology cannot distinguish between P.vivax and P. falciparum infections active surveillance by smear examination should continue (Kumar et al, 1987).

The following serological methods have been applied in epidemiology of malaria :

1. Enzyme linked immuno-sorbent assay (ELISA)
2. Indirect Immuno-fluorescence test (IIF)
3. Indirect haemagglutination test (IHA)
4. Radio Immunoassay (RIA)
5. Latex agglutination test
6. Gel immunoprecipitation test
7. Counter current immuno-electrophoresis (CIEP)
8. Complement fixation test.

9.1 Enzyme linked immuno-sorbent assay :

The generic term enzyme immuno-assay are generally known as Enzyme linked immuno-sorbent assay (ELISA). There are two widely accepted assays that employ labelled antibodies and antigens. They are immuno-fluorescence, in which a fluorescent dye is conjugated to the antibody, and radio-immunoassay, in which isotopes are attached to antibodies or antigens. Both assays are complex and can be performed only in few large centres.

The introduction of enzyme immunoassays, pioneered by Engvall & Perlmann (1971) offered an attractive alternative by using enzyme labelled antibody or antigens. The range of application of enzyme immuno assays, is potentially as wide as that of radio-immunoassay (Bull. W.H.O., 1976). The tests though evaluated in different laboratories may not be applicable in the field for diagnosis of malaria at present movement. However, it is envisaged that with the availability of different specificities of monoclonal antibodies by way of hybridoma technology and also with the help of recombinant DNA techniques immuno-diagnosis of malaria in the field situation may become a reality. Today, precipitation tests and radio-immuno-assays are rarely used, the former because of their sensitivity, the latter, because they have almost completely been replaced by ELISA (W.H.O. Immunodiagnosis in Malaria, Unpublished document, WHO/ Mal. 185, 1018, 1985).

Field applications :

For malaria the test was used by Voller et al (1974 a). Since then the test has been used in large number of studies of malaria (Ambroise Thomas et al, 1978; Adriason et al, 1979; Mahajan et al, 1981; Srivastava, et al 1983, 1981; Ray et al, 1983a, b; Dutta et al, 1982, 1984; Spencer et al, 1979, 1981; Voller et al, 1974 b, 1975, 1978, 1980; Kumar et al, 1986).

Merits :- This is a simple technique requiring a limited amount of maternal antigen which can be fixed on variety of solid supports from multi-well plastic plates to nitro-cellulose paper.

By using purified and defined material antigens in the ELISA, it proved possible to measure in a reproducible way the antibodies against asexual blood stages of P. falciparum in children to increase the sensitivity and specificity and to standardise the method. It can be automated for use in central laboratories where large numbers of samples have to be processed and the results may be quantitated. It may be used under field conditions where the test is semi-quantitative and can be read visually.

Demerits :- The main limitations are inter-laboratory variation due to difficulties in standardization and the relatively poor specificity and sensitivity of the ELISA when parasitized red blood cells extracts are employed for the coating of solid support. The method can be improved by the use of purified antigens (W.H.O. Bull. 65, 1986).

9.2 Indirect Immunofluorescence Antibody (IIF) Test :

This test was introduced by Coons et al (1942). Since then it has been intensively used in sero-diagnosis of many parasitic and microbial diseases. Brooke et al

(1959) detected P. berghei antibodies by this test and opened a new chapter in epidemiology of malaria.

Washed infected red blood cells used as antigen. Serum containing antibodies is incubated with antigen. The antigen antibody complex is coupled to a fluorescein labelled antisera and slides are examined by fluorescence microscopy.

The community used antigens have been obtained from patients affected by P. falciparum cultures and monkey blood affected by P. knowlesi, P. cynomolgi and P. coatneyi.

Field Application :

IIF test has been used in large number of studies (Ambroise Thomas et al, 1972, 1974; Thomas et al, 1975; Agarwal et al, 1981, 1982; Bruce-Chawatt et al, 1973, 1975; Meuwissen et al, 1974; Collins et al, 1966, 1967, 1971; 1972; Kagan et al, 1981; Voller et al, 1968, 1974; Sulzer et al, 1969, 1975; Mahajan et al, 1981; Gupta et al, 1981; Warren et al, 1975, 1976; Reay et al, 1973; Hornstein et al, 1983; Ray et al, 1983; Hall et al, 1978).

Srivastava et al (1983) observed its high diagnostic value since 98 percent of slide positive malaria patients carrying P. falciparum or P. vivax could be diagnosed. Furthermore positivity observed in

The preparation of comparable batches of antigen is relatively simple. The whole infected cell, morphologically identifiable, is used as antigen. The results of the test can be used to show differences in malaria endemicity between localities, and to detect transmission. A higher titres, the test is virtually always specific for malaria and sometimes can be used to indicate species prevelence. Any laboratory with facilities for carrying out IIF test for other diseases can perform the test for malaria if the antigen is provided.

Malaria parasite carriers can occasionally give negative reactions. This has been especially with children. The necessity for specialised equipment and personel limits, this test to major laboratories. Antigens are available from only a few centres and their storage requires considerable refrigeration space. The transport of antigen can present problems.

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MATERIAL AND METHODS

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1. The study area :

The present study was conducted in 20 villages namely - Pahari, Mirona, Maheba, Dhewani, Baral, Dakuan, Babri, Moreta, Ghushwan, Gulara, Mod Khurd, Mod Kalan, Sant-Bahta, Kibi, Bajehera, Bangra, Simthiri, Jaryai, Chhirona and Sultantpura, located within the area of Primary Health Centre (P.H.C.) Chirgaon which is the rural health training centre (R.H.T.C.) of the Department of Social and Preventive Medicine, Maharani Laxmi Bai Medical College, Jhansi (U.P.). The centre is being utilised for field training of undergraduate students in community health and for epidemiological researches.

1.1 Topography :

District Jhansi of Bundelkhand region situated in south-west of Uttar Pradesh, is surrounded by districts of Gwalior, Datiya, Shivpuri and Tehamgarh of Madhya Pradesh and Lalitpur, Hamirpur and Jalaun of Uttar Pradesh. P.H.C. Chirgaon is situated on Bombay-Kanpur road at a distance of 25 kms. from M.L.B. Medical College, Jhansi, U.P. It renders health care delivery to the population of 120 villages besides Chirgaon town where centre is located. Majority of study villages are

connected by pucca roads with the centre; a few, however are not approachable by easy means.

The geographical area of Community Development Block Chirgaon is 55.255 hectare constituting mainly of Padua soil which is suitable for wheat cultivation.

1.2 Climate :

Climate of the area is hot and dry. Mean monthly maximum and minimum temperature ranges between 47.1°C to 3.7°C respectively (1986-1987). General and real rainfall was recorded as 879 mm and 586 mm respectively during the calender year 1986. Mean monthly relative humidity ranges between 15% to 76% at 0730 hrs and 26 to 84% at 1530 hrs (Statistical Diary, U.P., 1987).

1.3 Population composition :

P.H.C. Chirgaon has a population of 1,08,561, according to 1981 census. The density of population is 1.96/hectare. Male : Female ratio is 1000 : 818. The literacy rate is 1 : 6 higher in comparison to Uttar Pradesh and 7.17% lower in comparison to whole of India (Census, 1981). Majority of them are Hindus followed by Muslims and then others (Govt. of U.P., 1986). Agriculture and labour are main occupations of the area.

1.4 Environmental conditions :

Mostly, houses are either kutcha or semi-pucca with a little or no facility of cross-ventilation. Open and insanitary wells are main source of water supply. There are no sewage and drainage system for disposal of excreta and waste water respectively.

Incidence of malaria was however not uniform throughout the block. These are foci of high and low incidence. The estimated A.P.I. of these villages was over 2.6 per thousand population as reported by District Malaria Office, U.P. (District Malaria Office, Jhansi).

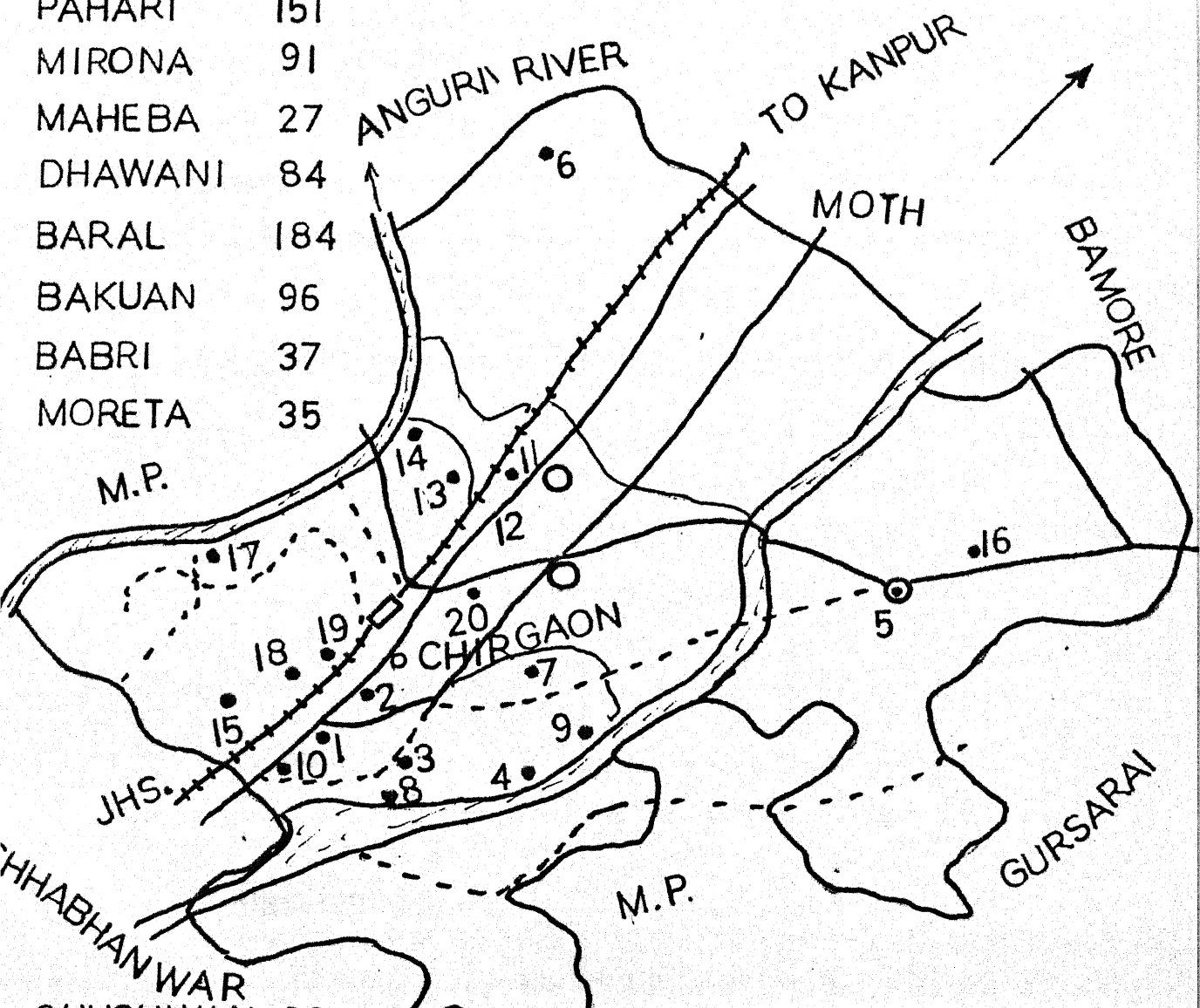
Annual parasite incidence and slide positivity rate in Chirgaon block during the year 1982-89 is as follows :

Year	A.P.I.	S.P.R.
1982	12.05	12.17
1983	13.01	12.16
1984	7.32	8.39
1985	7.99	8.16
1986	6.80	8.17
1987	2.96	2.89
1988	2.02	2.28
1989	1.04	1.10

Source : District Malaria Office, Jhansi (U.P.).

FIG. 3.1.

NO. OF SAMPLES COLLECTED FROM EACH VILLAGE (1-20)



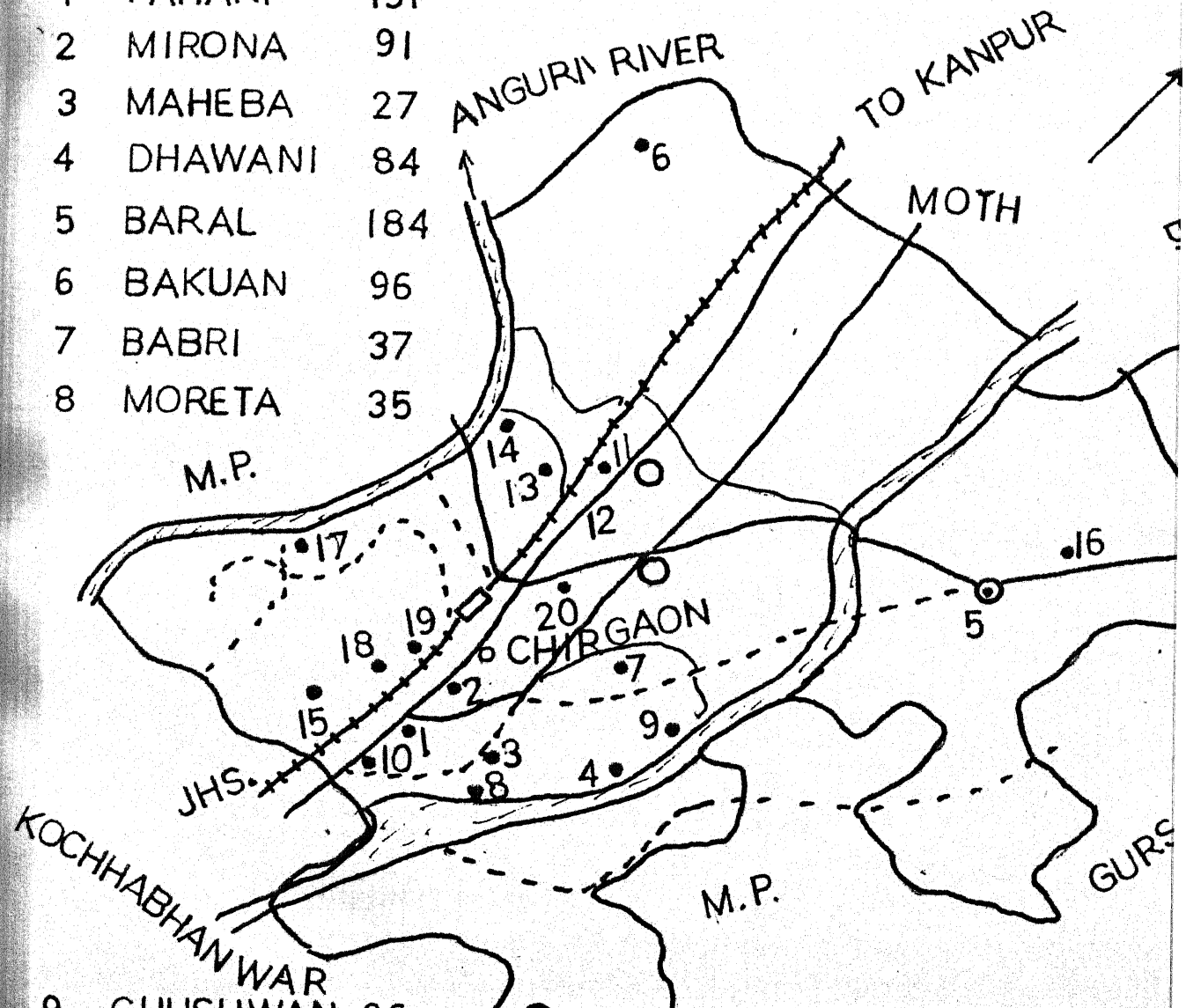
PAHARI	151
MIRONA	91
MAHEBA	27
DHAWANI	84
BARAL	184
BAKUAN	96
BABRI	37
MORETA	35

GHUSHWAN	26
GULARA	46
MOD KHURD	34
MOD KALAN	54
SANT BEHTA	28
NIBI	38

15	BAJHERA	65
16	BANGRA	80
17	SIMTHARI	92
18	JARYAYI	87
19	CHHIRONA	156
20	SULTANPURA	98

FIG. 3.1.

NO. OF SAMPLES COLLECTED FROM EACH VILLAGE (1-20)



1	PAHARI	151
2	MIRONA	91
3	MAHEBA	27
4	DHAWANI	84
5	BARAL	184
6	BAKUAN	96
7	BABRI	37
8	MORETA	35

9	GHUSHWAN	26
10	GULARA	46
11	MOD KHURD	34
12	MOD KALAN	54
13	SANT BEHTA	28
14	NIBI	38

15	BAJHERA	65
16	BANGRA	80
17	SIMTHARI	92
18	JARYAYI	87
19	CHHIRONA	156
20	SULTANPURA	98

2. Study design :

The population survey was carried out once during the transmission period (September and October, 1987). In this district, there was marked increase in the transmission level during the period September to October as observed by Srivastava et al (1975). The maximum prevalence is from August to November in most of the parts of India.

Seeing the paucity of time and limited resources available, it was thought to conduct the study in transmission period only. The number of samples collected from each village is shown in Figure 3.1.

2.1 Unit of study :

Households of every tenth house selected by systemic random sampling was the unit of study.

2.2 Sampling size and sampling :

In this study every tenth house was the unit of study. All individuals of a household were taken into study irrespective of their age, sex and health status except for infants under six months of age, who were not included to avoid the effect of maternal antibodies on the results of sero-epidemiology.

Twenty percent villages in the area under study were selected using simple random sampling method. This was done to provide a 20% sampling of the population of villages under study with inclusion of all age groups. The selection of villages in the block was done by simple random sampling method using table of random numbers (Fisher & Yates, 1957).

The family records of these selected villages as maintained by respective ANM's and CHV's in Chirgaon block, verified and made uptodate by making necessary alterations and additions during household listing in the selected villages. The records were re-verified at the time of sampling. The door to door survey was carried out by visiting once during the transmission period (September & October, 1987).

Heads of families of selected household were interviewed on a pre-tested schedule (see Appendix I) to collect information regarding various bio-social characteristics. Thereafter each individual of household was interviewed separately and information were recorded on a separate schedule (Appendix II). Every individual was examined clinically to find out any associated illness and to assess organomegaly.

2.3 Collection, transportation and storage of samples :

The blood samples of each individual with family

by door to door visit. By finger prick method, two spots of 2 cm. size were taken on Whatman's No. 3 filter paper strips. A thin and thick smear of individual was also prepared. The filter paper strips were air dried in shade. Dried samples were sealed in polythene bags and were transported to the laboratory in ice. In the laboratory, the filter paper strips were stored at -20°C until final analysis. The slides were fixed in methanol on the same day and stained with Geimsa stain. Later, they were examined under oil immersion lens of binocular compound microscope.

The collection and staining of glass slides were performed in usual manner (W.H.O., 1961). The blood films were stained with Geimsa Stain and examined for malarial parasites.

3. Performance of Serological Test :

3.1 Antigen :

P. falciparum antigen was prepared from in-vitro culture of P. falciparum maintained at National Institute of Communicable Diseases (NICD), Delhi. Test was essentially performed as described by Hall et al (1978) and some modifications suggested by Ray et al (1983). The parasite was at a sub-culture level of 251 and contained approximately 8-9 percent parasitaemia with mainly schizonts. The antigen was prepared by saponin

treatment of the culture followed by sonication. Antigen was schizont antigen and was more than 90% pure.

3.2 Reference sera :

The positive reference serum was obtained from a person having heavy malaria infection. The negative reference serum was a pool from slide negative apparently healthy human beings. These had previously been tested by the IIF & ELISA.

4. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

a) The Micro-ELISA test was performed in the 96-well flat bottom polystyrene Micro-ELISA plate (Nunc. Micro titre No. 1 were used as carrier surface for antigen.

b) Enzyme Conjugate :- Anti-human IgG (heavy and light chains) labelled with horse radish peroxidase was obtained from Cappel Laboratories (Cochranville. U.S.A.)

c) Substrate :- Enzyme substrate ortho-phyenylene diamine (O.P.D.) was obtained from Sigma Chemical. U.S.A. for measuring peroxidase activity.

d) A reference positive and reference negative serum were used for determining optimal dilution of antigens, serum and conjugate using in-vitro culture P.falciparum antigen dilution from 1 : 3000 and serum dilution ranging from 1 : 400 in P.B.S. Tween-20.

The optimum antigen dilution using in vitro culture *P. falciparum* antigen that gave a strong reading with positive serum and low reading with similar dilution of negative serum was found 1 : 3000 and serum dilution was 1 : 400. The optimal conjugate dilution was determined by chequer board titration. Conjugate dilution (1 : 1500) was found to be optimum.

Micro-ELISA Procedure :- Test was performed according to method described by Ray et al (1983).

(i) Sensitisation of Micro-titre plate :

Antigen was diluted to optimum concentration in coating carbonate buffer (0.06 M, PH 9.6). 200 ul each of the optimally diluted antigen was added into the wells of a micro-titre plate. The plates were covered and kept in plastic box to incubate at 4°C for 18 hrs.

Dilutions (1 : 200 ul) of the test and control sera were made in P.B.S. pH 7.2 containing 0.05% Tween-20 (see Appendix XXI). The antigen sensitized plates were took out from the refrigerator and the excess liquid was removed from the plate. The plate was washed twice in same buffer for 5 minutes each time and then dried.

(ii) Incubation with sera :

The antigen coated wells were filled with test and control sera of 200 ul on the washed antigen sensitized plate. Antigen control was kept adding P.B.S. Tween-20 only.

The plates were covered and incubated at room temperature inside the wet plastic box for one hour. The wells were washed for 5 minutes thrice with P.B.S./T to remove unbound serum.

(iii) Incubation with Conjugate : Each of the well was then filled with 200 ul vol. of optimal diluted (1 : 1500) conjugate and then incubated in a humid chamber at room temperature for one hour. After incubation unbound conjugate was removed with washing three times P.B.S./T for five minutes each.

(iv) Substrate reaction : Peroxidase bound to the wells was finally estimated by adding 200 ul of substrate solution in each well and incubating the plate at room temperature in the dark. The reaction is allowed to proceed for 10-15 minutes. The reaction was stopped with the addition of 50 ul of 5N H_2SO_4 in each well.

For expression of results, the reading at a dilution of 1 : 400 was used since at this dilution, the positive-negative differentiation was best. 93.4 percent of the control sera from Delhi and all the sera from Kashmir gave a negative ($\angle$ 0.4) reading. Taking this as the point of differentiation between the positive and negative sera, 32.30 percent of our study individuals showed reaction upto 0 - 0.4 O.D. (E_{492}); 24.01 percent showed 0.4 - 0.6 O.D. reaction (E_{492}); 14.01 percent

individual showed 0.6 - 0.8 O.D. (E_{492}) and 6.05 percent showed 0.1 - 1.0 O.D. (E_{492}) reaction. All the slide positive individual showed more than 1.0 O.D. (E_{492}) reaction.

Reading was taken at optical density (OD) at wave length of 492 n.m. using spectronic 20 spectrophotometer

5. Indirect Immunofluorescence Test :

A local strain of P.falciparum (PAN-5) was adopted to continuous culture and maintained in NICD Laboratory since 1978 by the method of Rai Chowdhuri et al. At sub-culture level of 122 when the parasitaemia was 8% with rings (20 percent), trophozoites (35 percent) and schizont (45%), the culture was washed five times in P.B.S. pH 7.2. After the final washing, it was suspended in the same buffer in such a way so as to contain about 20-30 plasmodia per high power field in a thick smear (Sulzer et al, 1969). While preparing the smears, care was taken that the cells did not settle out of the antigen suspension in the pasteur pipettes. After drying, the smears were stored at -70°C, wrapped in wax papers.

5.1 Reference sera :

The reference malaria positive sera from malaria cases and negative sera from non-malarious area were received from C.D.C. Atlanta and stored as above.

5.2 Fluorescent Conjugate :

Anti-human IgG (heavy and light chains) labelled with fluorescein isothiocynate was obtained from immuno-diagnostic limited. Different conjugate dilutions were tested for finding the optimum dilution to be used. It was found that conjugate dilution 1 : 10 was giving highest titre with reference positive and lowest with reference negative sera.

5.3 Performance of the Test :

The test was carried out essentially by the method of Sulzer et al (1969) with some modifications suggested by Ray et al (1982). The antigen slides (stored at -70°C) were taken out and kept on racks made in glass petridishes and were labelled and allowed to dry. The test sera along with positive and one negative control sera were diluted in two fold dilution starting from 1 : 32 to 64 in P.B.S. pH 7.2. A drop of each dilution of test sera was placed covering each antigen smear. A control smear was kept receiving P.B.S. pH 7.2 instead of serum. The slides were placed inside humid petri dishes and incubated at 70°C for 30 minutes. Next the slides were washed thrice (each time for 10 minutes with P.B.S. pH 7.2) with manual stirring and dried quickly under the fans.

Optimal dilutions (1 : 40) of commercial anti-human IgG, A and M (H and L) conjugated with fluorescein isothiocynate (Institute Pasteur Production) was added to cover the smear fully.

Incubation, washing and drying in the above manner followed. The slides were mounted with buffered glycerol (pH 7.2) and examined under a fluorescent microscope.

5.4 Reading and interpretation of Results :

Fluorescence was subjectively graded from negative to 4+ and ++ and above were considered positive. The fluorescence of the parasites were seen against a background of faintly visible erythrocytes (Ray et al. 1982).

6. Compilation, Tabulation and Interpretation of Data :

Data so obtained from the study was subjected to critical statistical analysis which consist of estimation of the prevalence of antibody titre in random population and to find out, correlating it with various bio-social characteristics of the population.

The usual tests of significance such as Chi square test was used to determine the significance of the association between the two variables and difference between two parametric values.

7. Limitation of study :

The study had been carried out in partial fulfilment of the requirements of M.D.(Social & Preventive Medicine) examination and therefore suffers from limitations of time and resources. Many of the informations sought, are based on the capacity to recall, the limitations of which do not need any emphasis. The reluctance on the part of individuals in giving the blood samples proved a great difficulty in the course of study. Inspite of the best efforts made, such samples of all individuals could not be obtained.

To show seasonal variations in the transmission of the disease, the non-transmission survey could not be conducted due to paucity of time and resources available.

Due to unavoidable reasons and paucity of time, the IIF test could be performed only in ELISA positive proven samples of blood.

8. Different criteria adopted :

8.1 Family type :

Any family with husband, wife and their offsprings was considered as nuclear and rest were considered as joint.

3.2 Family size :

A family upto 5 members was considered as small, whereas one with 6 or more members was taken as large.

3.3 Social class :

Social classification of families used in this study was as given by Srivastava et al (1982). Criterion of social classification brought forth by Srivastava et al (1982) is given below :

Mean monthly per capita income	Social Class
Rs. 600/- and above	I
Rs. 300/- to Rs. 599/-	II
Rs. 140/- to Rs. 299/-	III
Rs. 60/- to Rs. 139/-	IV
Rs. 60/-	V

****

OBSERVATIONS

OBSERVATIONS

The present study was conducted at Primary Health Centre, Chirgaon, Jhansi (U.P.). The study was carried out in twenty villages. The systemic sampled population under study consisted of 1520 individuals of 269 families out of total 290 families.

1. Population under study :

There were 98.9% Hindus and 1.09 percent Muslims. Out of these, 15.53% individuals belonged to upper caste and 49.20% backwards and 25.27 percent scheduled caste. The majority of families were joint (75.29%) and rest belonged to nuclear (24.71%) families. The maximum percentage (32.71%) of families consisted of 5-6 members and minimum percentage (1.42%) of families consisted of 1-2 members. There were three-fourth families in social class IV (46.29%) and class V (28.25%) and one-fourth families classified in social class III (22.30%) and social class II (4.47%). The main occupation of the families was agriculture (71.92%) followed by labour (25.46%), service (2.0%) and rest were engaged in business and other occupations. In the study, married individuals were 59.74 percent and 37.56 percent unmarried and rest of them were widow/widower and divorcee. There were

64.54 percent illiterate individuals, followed by literate (19.81%), just literate (5.98%) and children (9.67%).

1.1 Male Female ratio :

The sex variation in the study revealed that adult male contributed 36.91 percent and adult female 31.37 percent. The paediatric population 0 - 14 years was 31.72 percent; male accounted for 18.03 percent and female 13.69 percent.

An attempt has been made in this study to see the relationship between the prevalence of malaria and its various bio-social characteristics. The elaborate description for this relationship has been given in subsequent text, taking the various variables one by one. The impact of these variables on the distribution of disease has been viewed separately amongst the studied population.

TABLE 1

Distribution of individuals by their age and sex.

Age (year)	Male		Female		Total	
	No.	%	No.	%	No.	%
< 1	1	0.07	-	-	1	0.07
1 - 4	63	4.14	41	2.70	104	6.84
5 - 9	103	6.78	95	6.25	198	13.03
10 - 14	107	7.04	72	4.74	179	11.78
15 - 24	174	11.45	147	9.67	321	21.12
25 - 34	134	8.82	112	7.37	246	16.19
35 - 44	96	6.32	81	5.33	177	11.65
45 - 54	70	4.60	80	5.26	150	9.86
55 - 64	61	4.01	44	2.89	105	6.90
65 +	26	1.71	13	0.86	39	2.57
Total	835	54.93	685	45.07	1520	100.00

Table 1 shows the age and sex distribution of study population.

The percentage of males and females was 54.93 and 45.07 respectively. It was observed that maximum (21.12%) individuals belonged to the age group 15 - 24 years, followed by 16.19 percent in age group 25 - 34 years, whereas, there was only one (0.07%) individual in age

group < 1 year. The paediatric population accounted for 31.72 percent.

2. Bio-social characteristics of population :

2.1 Age :-

TABLE 2

Slide positivity and sero-positivity according to age.

Age (years)	Total examined	Slide examination		Serological examination	
		No. found positive	Positivity rate (%)	No. found positive	Positivity rate (%)
< 1	1	-	-	-	-
1 - 4	104	4	3.85	20	19.23
5 - 9	198	1	0.50	33	16.66
10 - 14	179	3	1.68	37	20.67
15 - 24	321	6	1.87	134	41.74
25 - 34	246	11	4.47	158	64.22
35 - 44	177	7	3.95	126	71.19
45 - 54	150	3	2.00	106	70.66
55 - 64	105	4	3.81	79	75.24
65 +	39	1	2.56	29	74.36
Total	1520	40	2.63	722	47.50

(X^2=2.77, d.f.=2, P < 0.25) (X^2=308.1, d.f.= 8, P < 0.001)

FIG.- 1

BAR DIAGRAM SHOWING S.P.R. SEROPOSITIVITY RATES AMONGST INDIVIDUALS BY AGE

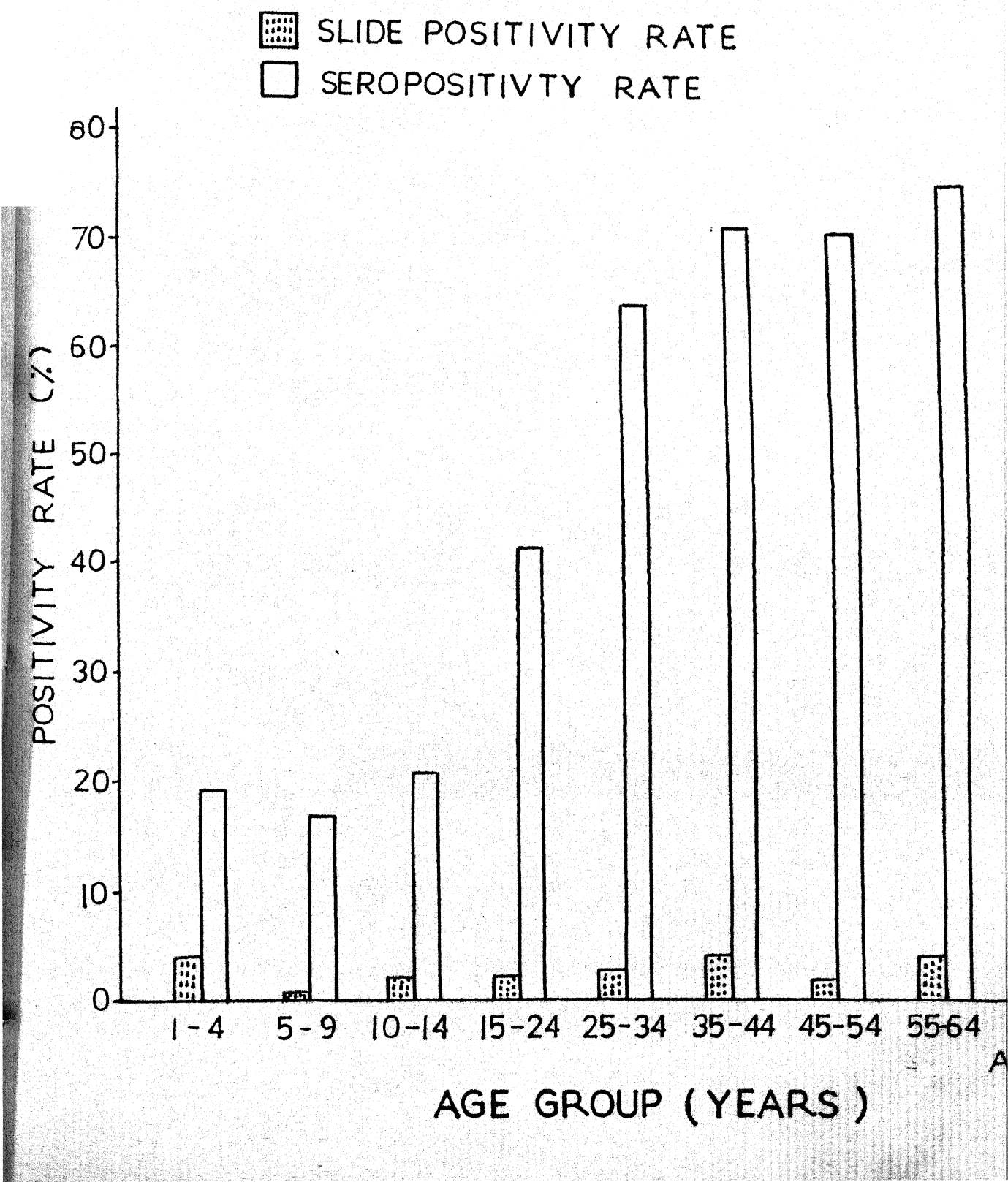


Table 2 and Fig. 1 shows that out of total 1520 individuals, 40 (2.63%) were slide positive for malaria. *P. vivax* infection was detected in all these cases. The slide positivity rate was highest (4.47%) in age group 25 - 34 years, followed by 3.95 percent in the age group 35 - 44 years. The higher (3.85%) positivity in age group 1 - 4 years shows that fresh transmission is occurring in this area, difference is not statistically significant.

The sero-positivity rate of 19.23 percent was seen in $\leq$ 1 - 4 years age group, 20.67 percent in 6 - 15 years age-group, 71.19 percent in 35 - 44 years age-group and 75.24 percent in 55 - 64 years and above age groups.

The sero-positivity was significantly low in those aged $\leq$ 1 - 14 years when compared with 15 - 44 years and 45 - 64 years age-groups.

FIG.-2

BAR DIAGRAM SHOWING SLIDE POSITIVITY AND SEROPOSITIVITY RATES AMONGST INDIVIDUALS BY SEX

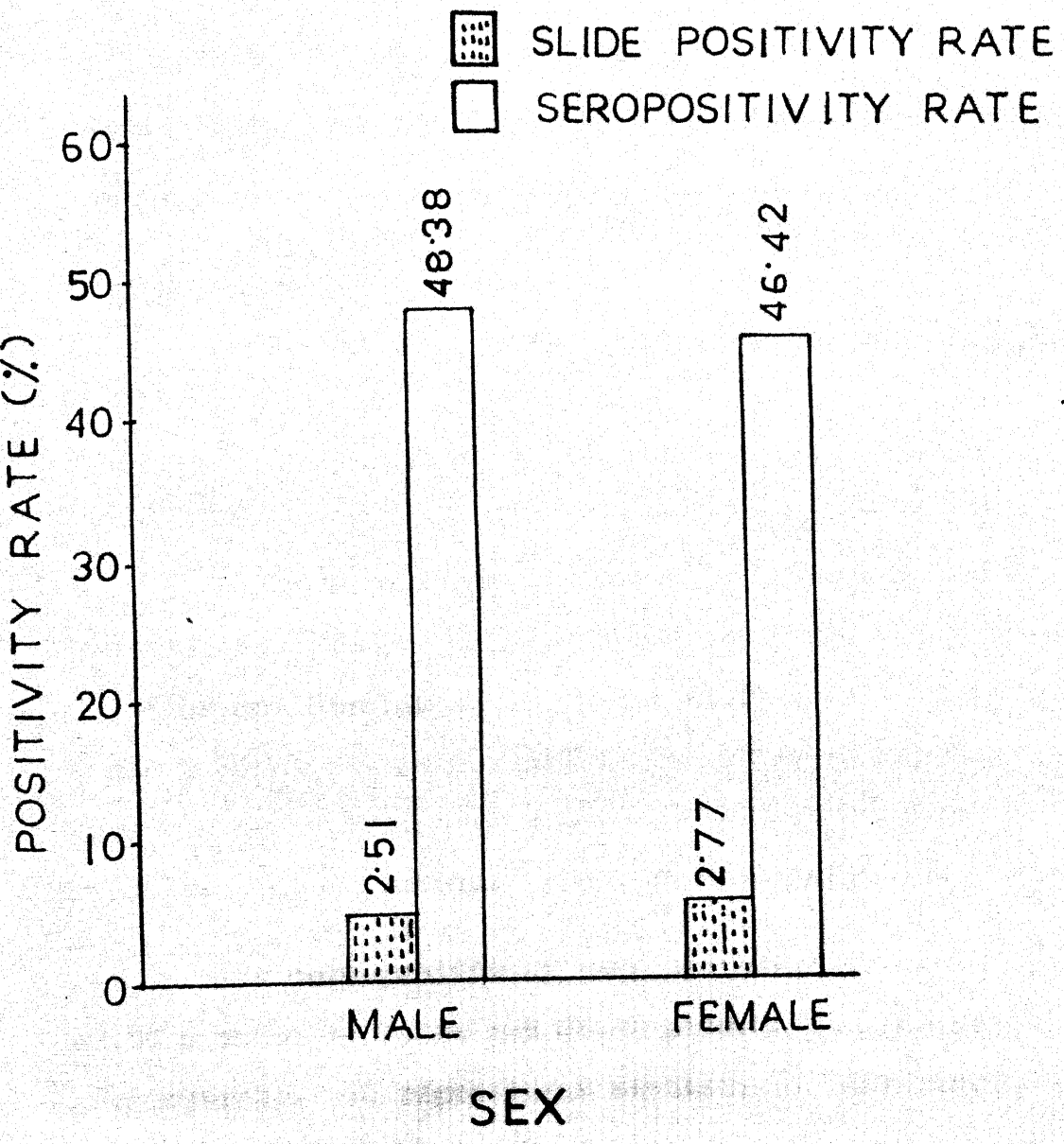


TABLE 3

Distribution of slide positivity and sero-positivity according to sex.

Sex	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate (%)	No. found positive	Positivity rate (%)
Male	835	21	2.51	404	48.38
Female	685	19	2.77	318	46.42
Total	1520	40	2.65	722	45.50

(x^2=0.1, d.f.= 1, P $>$ 0.75). (x^2=0.583, d.f.=1, P $>$ 0.25)

2.2 Sex :

Table 3 and Fig. 2 shows the distribution of the individuals according to their sex. The percentage of males and females is 54.93 and 45.07 respectively (male : female :: 1000 : 818). The slide positivity rate was 2.51 percent in males and 2.77 percent in females. The difference is not statistically significant.

The sero-positivity rate was observed as 48.38 percent in males and 46.42 percent in females respectively. No significant statistical difference was observed in both sexes.

FIG. - 3

BAR DIAGRAM SHOWING SLIDE POSITIVITY & SEROPOSITIVITY ACCORDING TO RELIGION AND CASTE

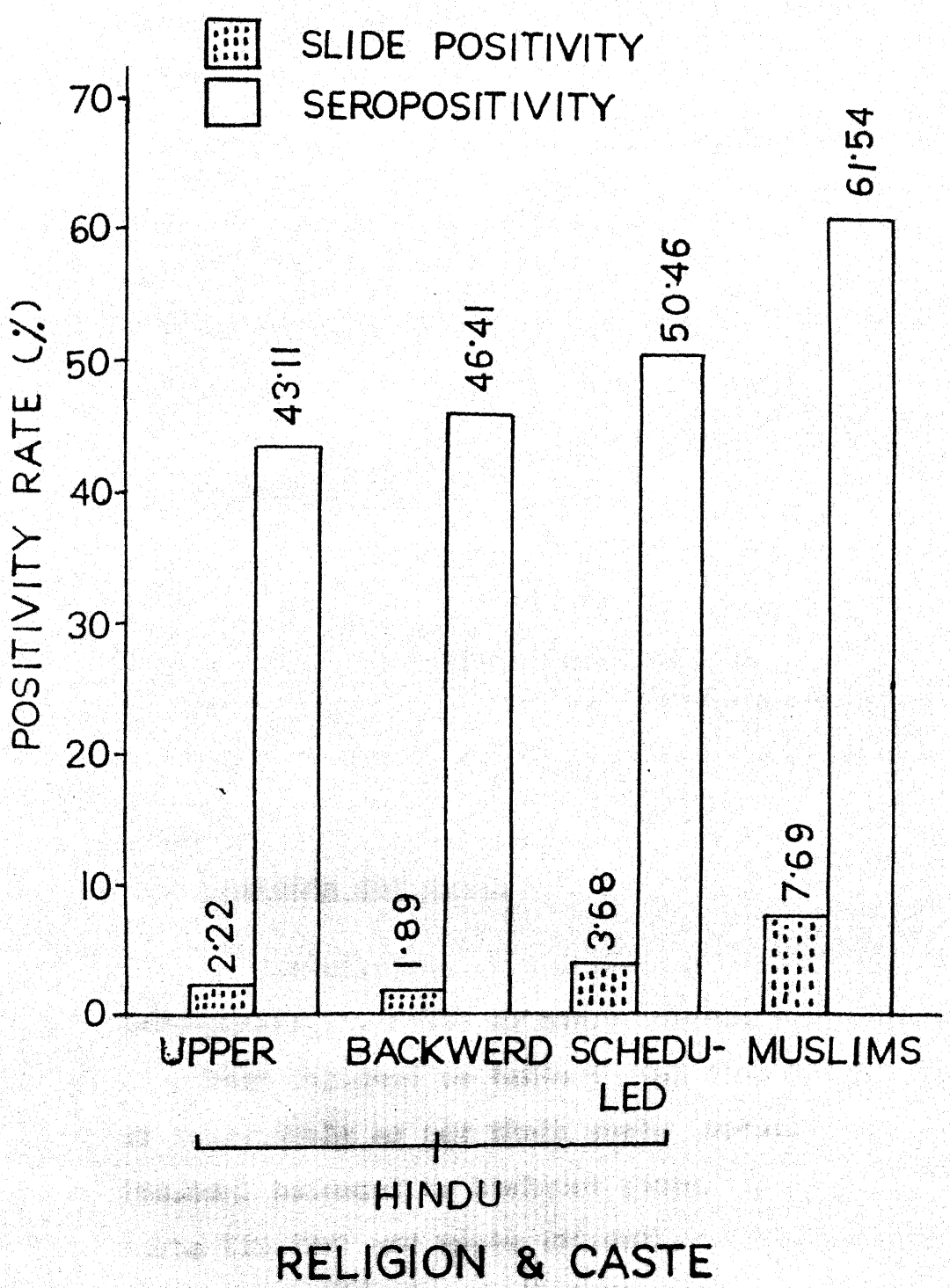


TABLE 4

Slide positivity and sero-positivity according to religion and caste.

Religion & caste	No.of cases	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Religion :					
• Hindu :					
- Upper	225	5	2.22	97	43.11
- Backward	739	14	1.89	343	46.41
- Scheduled	543	20	3.68	274	50.46
• Muslims	13	1	7.69	8	61.54
Total	1520	40	2.63	722	47.50

(χ^2=4.046, d.f.= 2, P $\gtrsim$0.10), (χ^2=4.008, d.f.=3, P $\gtrsim$0.25)

2.3 Religion and caste :

Distribution of individuals, slide positivity and sero-positivity rates by their various religions and castes, have been depicted in table 4 and fig. 3. The distribution of individuals as per their caste revealed that maximum (48.68%) belonged to backward caste, followed by scheduled caste (35.72%) and upper (14.80%). While calculating the positivity rate of disease in relation to caste, it was

observed that all individuals were Hindu except one slide positive case being Muslim.

However, the slide positivity rate was highest (7.69%) for Muslims, whereas for scheduled and backwards it was found to be 3.68 and 1.89 percent respectively.

The difference observed was, statistically not significant. The sero-positivity rate was also higher in Muslims (61.54%) and scheduled castes (59.46%) followed by backwards (46.41%), it was lower (43.11%) in upper castes. However, difference was, statistically insignificant.

TABLE 5

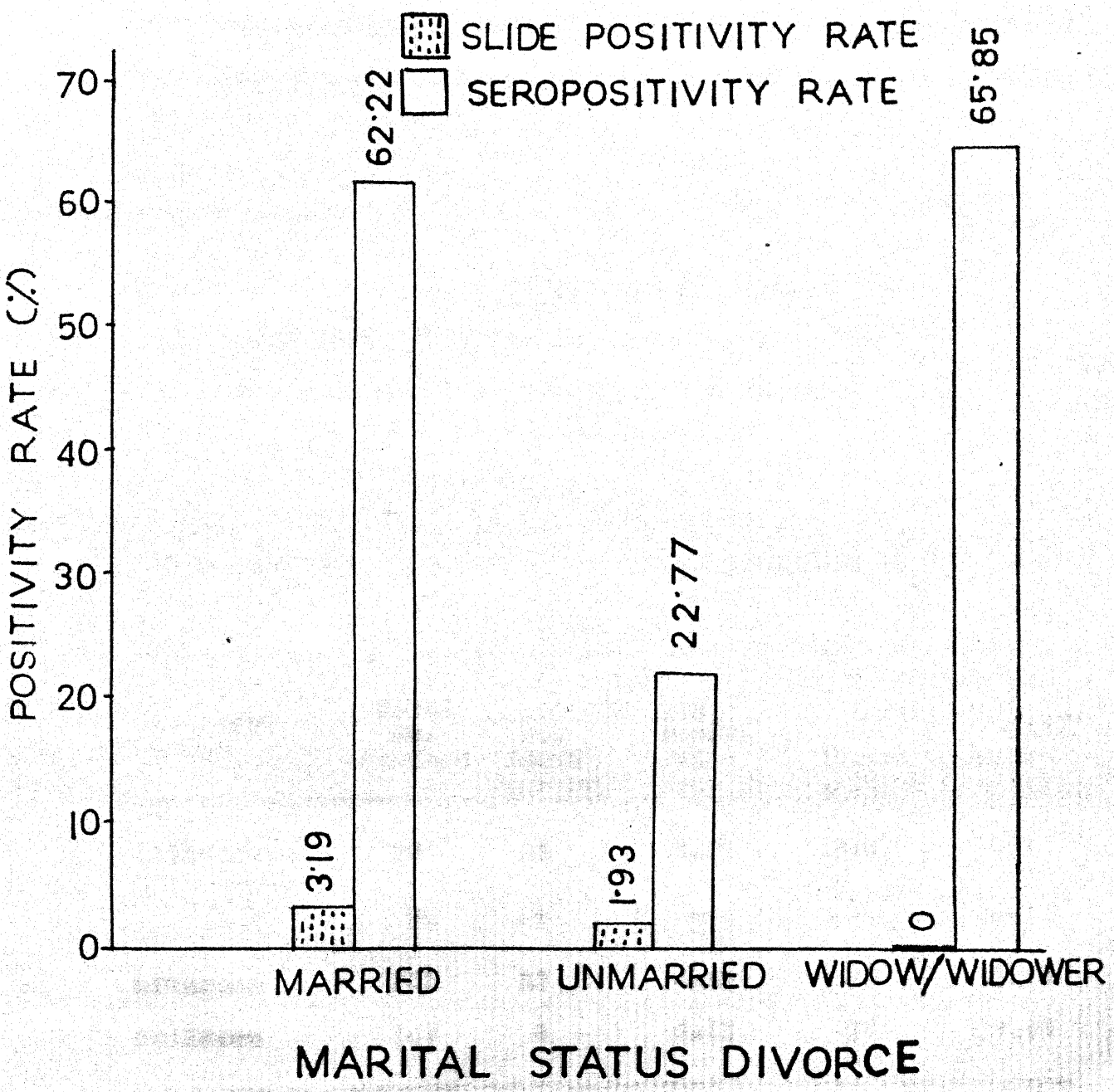
Slide positivity and sero-positivity according to their marital status.

Marital status	Total examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Married	908	29	3.19	565	62.22
Unmarried	571	11	1.93	130	22.77
Widow/ Widower/ Divorcee	41	-	-	27	65.85
Total	1520	40	2.63	722	47.50

(χ^2=8.95, d.f.=1, P $<$ 0.01). (χ^2=224.4, d.f.=2, P $<$ 0.001)

FIG. -4

BAR DIAGRAM SHOWING SLIDE POSITIVITY AND SEROPOSITIVITY AMONGST INDIVIDUAL BY MARITAL STATUS



2.4 Marital Status :

Table 5 & Fig. 4 shows distribution of individuals and positivity rates of malaria in relation to their marital status. The majority (59.74%) of individuals were married, followed by unmarried (37.56%). The widow/widower and divorcee were 2.69 percent. The slide positivity rate was higher (3.19%), in married and lower (1.93%) in unmarried individuals. The difference was statistically significant. The sero-positivity rate was higher (65.65%) in widow/widower and divorcee and in married (62.22%) and it was lower (22.77%) in unmarried individuals. The difference was statistically significant.

TABLE 6

Slide positivity and sero-positivity according to literacy status.

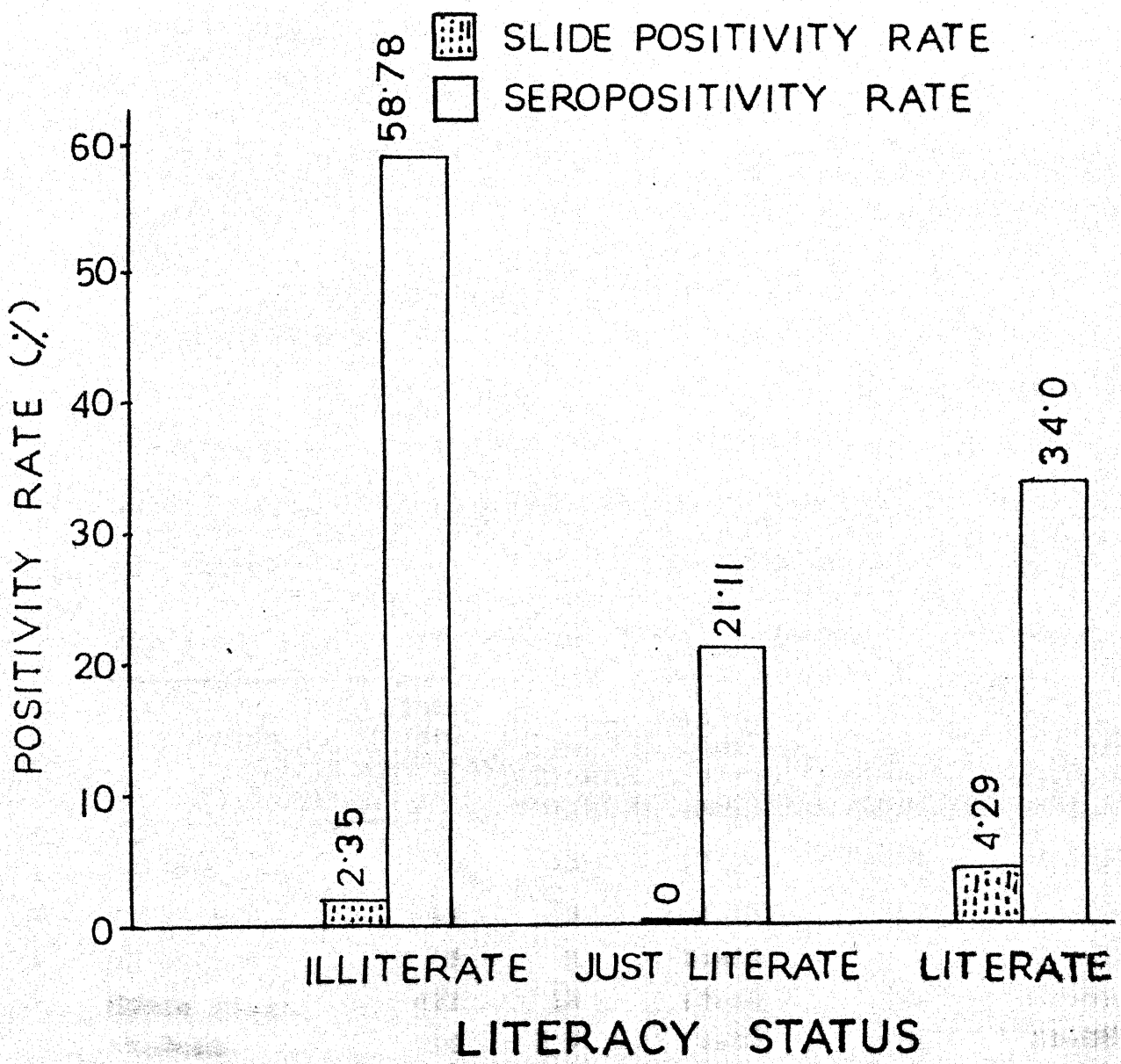
Literacy status	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Illiterate	980	23	2.35	574	58.78
Just literate	90	-	-	19	21.11
Literate	303	13	4.29	103	34.00
Children	147	4	2.72	24	16.33
Total	1520	40	2.63	722	47.50

(χ^2=4.126, d.f.=1, P $\gtrsim$0.025). (χ^2=191.691, d.f.=2, P$\lesssim$0.001)

FIG. -5

BAR DIAGRAM SHOWING SLIDE POSITIVITY RATE & SEROPOSITIVITY RATE AMONGST INDIVIDUALS BY LITERACY STATUS

(CHILDREN UPTO 5 YRS. WERE IGNORED IN FIG)



2.5 Literacy status :

Relationship between literacy status of individuals and slide positivity and sero-positivity have been shown in table 6 & fig. 5. The majority of individuals were illiterate (64.54%) or just literate (5.98%). Literate contributed only 10.80 percent.

The slide positivity rate of 4.29 percent was observed in literate, followed by children (2.72%). It was lower (2.35%) in illiterate. The difference was statistically significant.

The sero-positivity rate was highest (58.78%) in illiterate and lower (34.00%) in literate. It was further observed that sero-positivity rate declined with improvement in literacy status. However, it was statistically significant.

TABLE 7

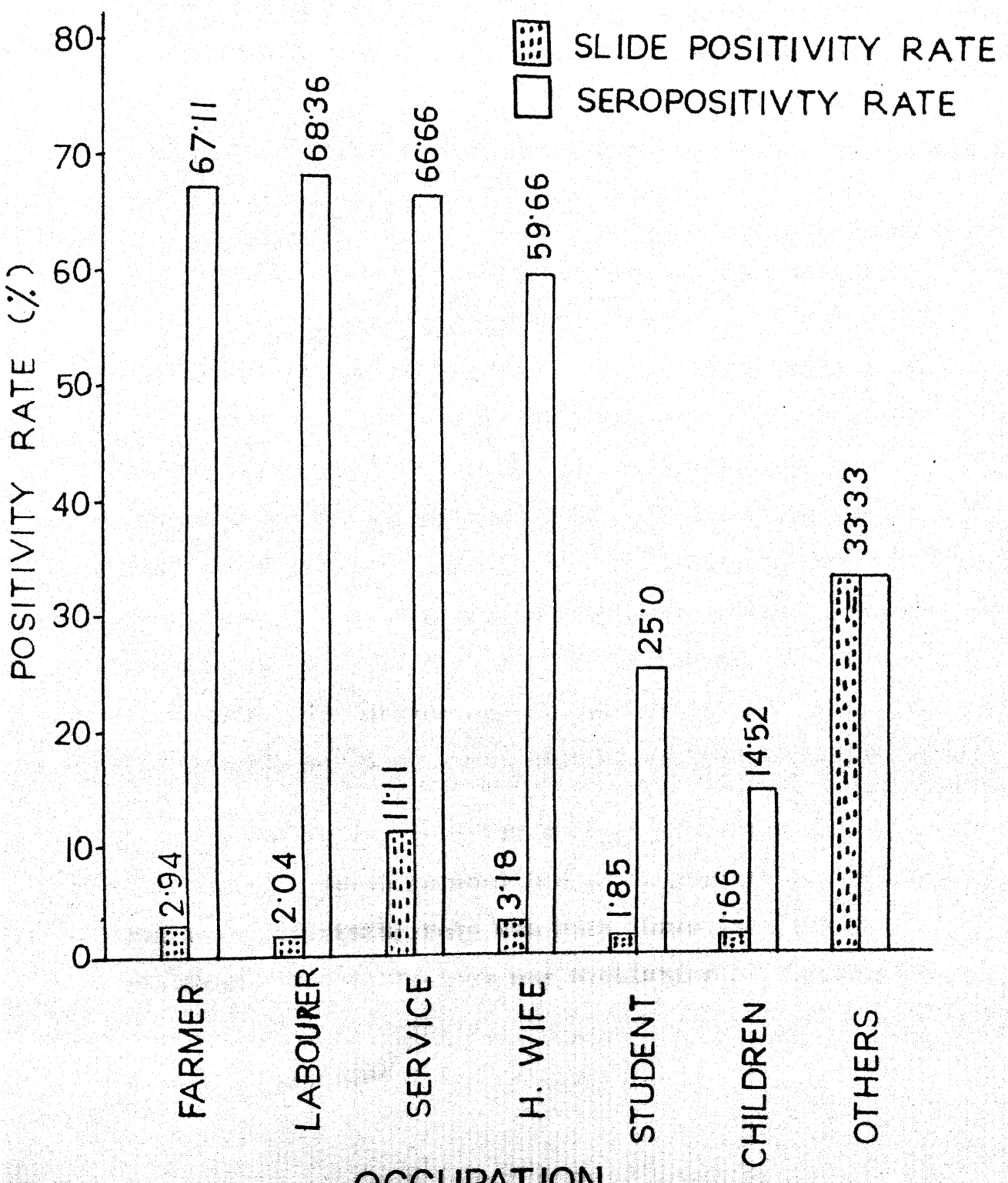
Slide positivity and sero-positivity according to occupation.

Occupation	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Farmer	374	11	2.94	251	67.11
Labourer	98	2	2.04	67	68.36
Service	9	1	11.11	6	66.66
House wife	471	15	13.18	281	59.66
Student	324	6	1.85	81	25.00
Children	241	4	1.66	35	14.52
Others	3	1	33.33	1	33.33
Total	1520	40	2.63	722	47.50

(χ^2=1.4172, d.f.=3, $P > 0.5$), (χ^2=243.927, d.f.=5, $P < 0.001$)

FIG.-6

BAR DIAGRAM SHOWING S.P. R. & SEROPOSITIVITY INDIVIDUALS BY OCCUPATION



3.6 Occupation :

Table 7 & Fig. 6 give the slide positivity rate and sero-positivity according to occupation. Farming (24.61%) and labourer (6.45%) were the predominant occupation in the study, followed by housewives who contributed 30.99 percent. There were 21.32 percent individuals amongst students whereas 15.86 percent accounted for children.

The slide positivity rate was higher (11.11%) individuals engaged in service. Most of them were employed in Parichha Thermal Power Project, while studying population for slide positivity as per their occupation it was recorded that it was higher (2.94%) amongst farmer's and/or housewives (3.18%), followed by 2.04 percent in labourer. Children showed lower slide positivity rate. However, a very high (33.33%) slide positivity rate was observed in individuals grouped as others. The difference between slide positivity rate for various occupation was statistically not significant.

The sero-positivity rate in labourers and farmers were 68.36 percent and 67.11 percent respectively. The sero-positivity rate was much lower (14.52%) in children. The difference was statistically significant.

FIG.-7

BAR DIAGRAM SHOWING S.P.R. & SEROPOSITIVITY RATE AMONGST INDIDIVIDUALS BY SOCIALCLASS

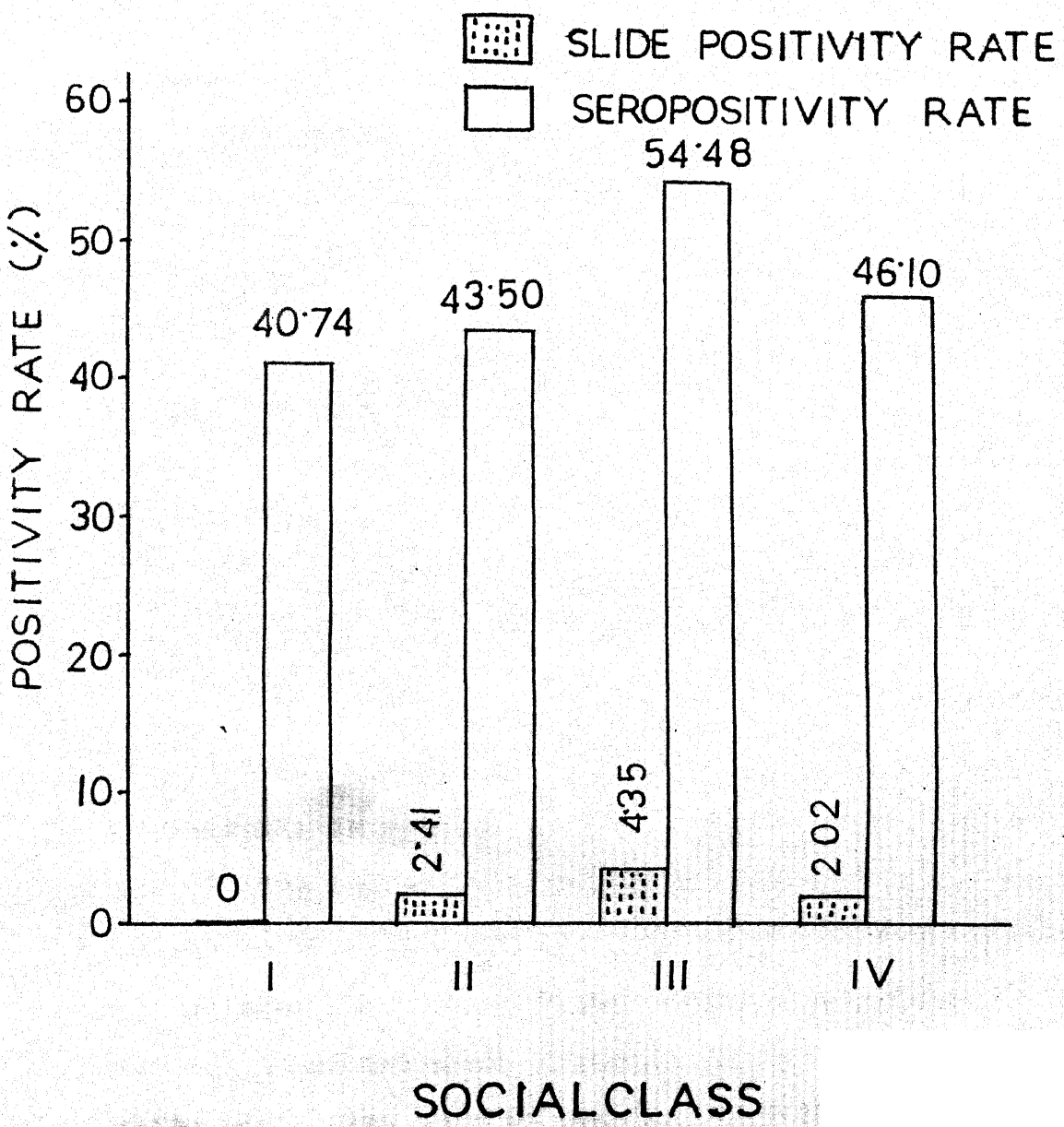


TABLE 8

Slide positivity and sero-positivity according to social class.

Social class	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
II	54	-	-	22	40.74
III	331	8	2.41	144	43.50
IV	391	17	4.35	213	54.48
V	744	15	2.02	343	46.10
Total	1520	40	2.63	722	47.50

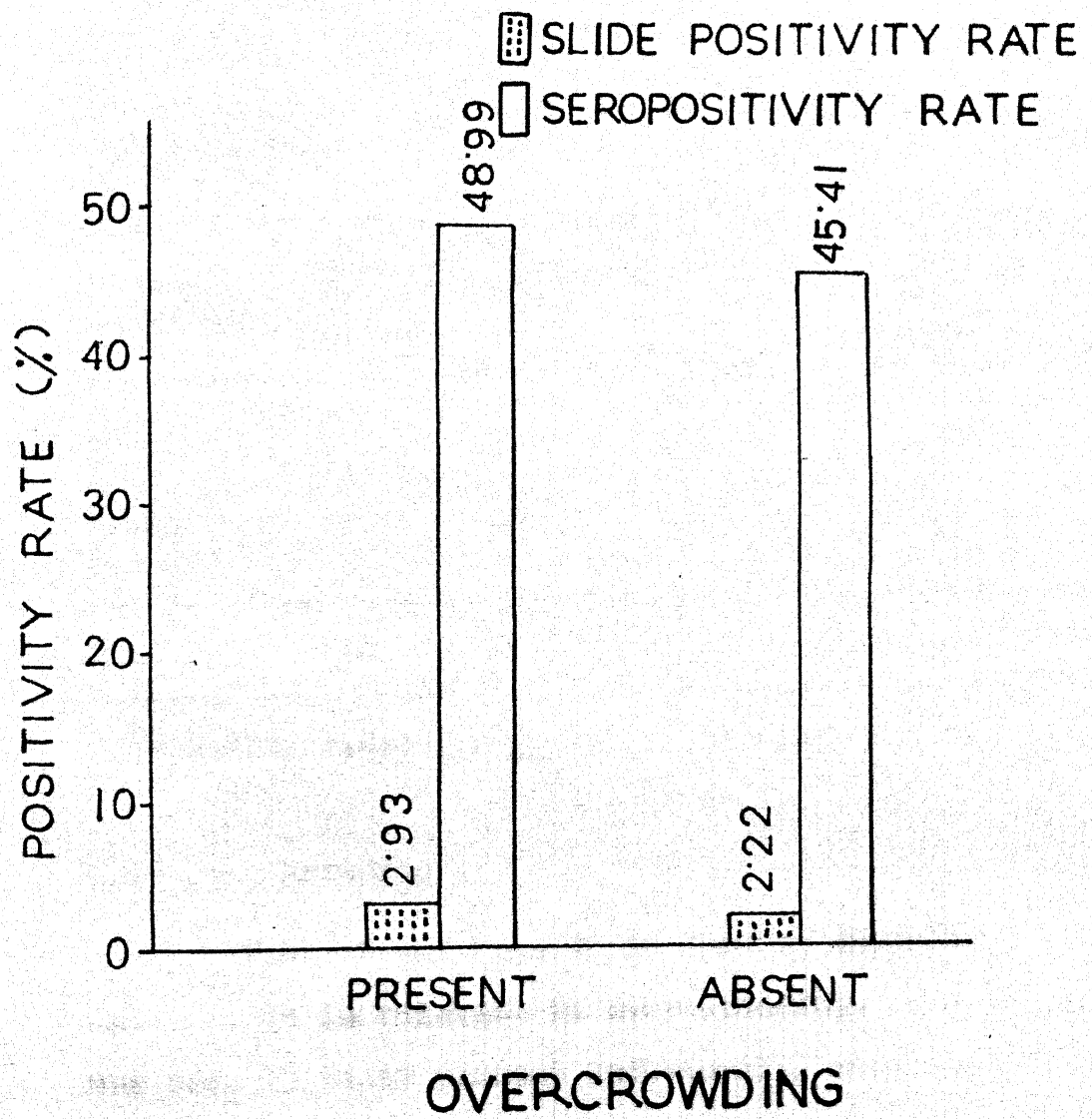
Note: There was no family from Social Class I.
(χ^2=7.125, d.f.=3, P $\gtrless$0.05). (χ^2=11.242, d.f.=3, P $\gtrless$0.010)

2.7 Social Class :

Table 8 and Fig. 7 give distribution of individuals in various social classes of population. Out of 1520 individuals, maximum (48.95%) were belonging to social class IV, followed by social class V (25.72%), social class III (21.78%) and social class II (3.55%). No individual belonged to social class I. While calculating slide positivity rate in relation to various social classes, it was observed that slide positivity rate was higher in social class V (4.35%) in comparison to social class IV

FIG.-8

BAR DIAGRAM SHOWING S. P. R. & SEROPOSITIVITY RATES AMONGST INDIVIDUALS BY OVERCROWDING



(2.02%) and social class III (2.41%). Yet the difference was statistically not significant. The sero-positivity rate was also higher in social class V (54.48%) followed by social class IV (46.19%) and lower in social class II (40.74%). However, the difference was statistically significant.

TABLE 9

Slide positivity and sero-positivity according to over-crowding.

Over-crowding	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Present	888	26	2.93	435	48.99
Absent	632	14	2.22	287	45.41
Total	1520	40	2.63	722	47.50

(χ^2= 0.719, d.f.=1, P $>$ 0.25). (χ^2=490, d.f.=1. P $<$ 0.010)

2.8 Over-crowding :

Table 9 and Fig. 8 is showing distribution of individuals in relation to over-crowding. Over-crowding was seen in 58.42 percent individuals, whereas 41.58 percent individuals were residing in sufficient number of living rooms. The slide positivity rate was almost equal in both groups whether over-crowding was present or not.

Slide positivity rate for individuals living under-crowded and un-crowded conditions were 2.93 percent and 2.22 percent respectively; the difference being statistically insignificant.

The sero-positivity rate for individuals living in crowded and uncrowded dwelling were 48.99 and 45.41 percent respectively. The difference being statistically insignificant.

TABLE 10

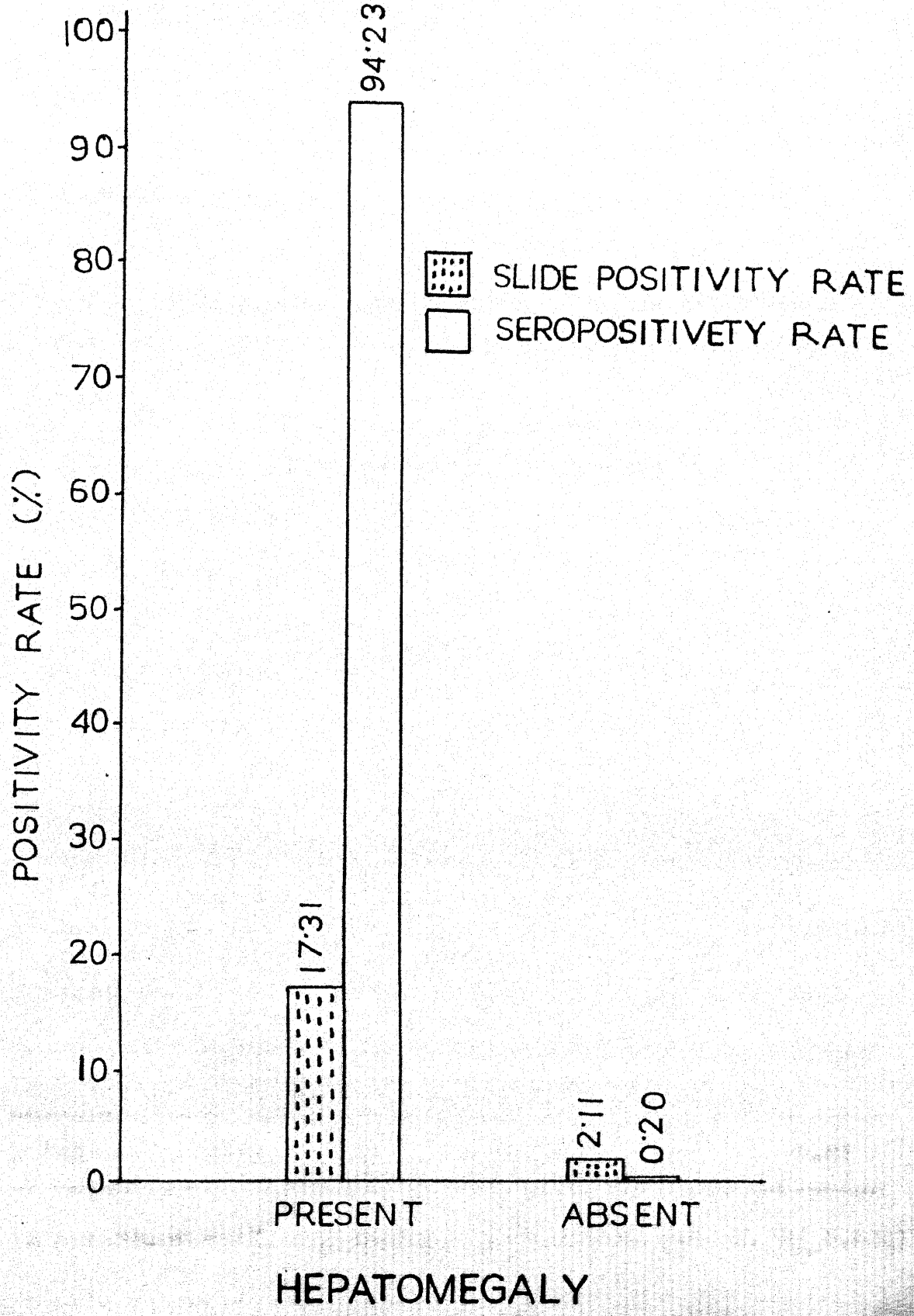
Slide positivity and sero-positivity according to hepatomegaly.

Hepato-megaly	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Present	52	9	17.31	49	94.23
Absent	1468	31	2.11	3	0.20
Total	1520	40	2.63	52	3.42

(χ^2=43.93, d.f.=1, $P < 0.001$). (χ^2=1328.02, d.f.=1, $P < 0.001$)

FIG. - 9

BAR DIAGRAM SHOWING S.P.R. & SEROPOSITIVITY RATE AMONGST INDIVIDUALS WITH HEPATOMEGALY AND WITHOUT HEPATOMEGALY



3. Slide positivity and sero-positivity in relation with clinical manifestations :

3.1 Hepatomegaly :

Table 10 and Figure 9 shows, that out of 1520 individuals, 3.42 percent showed hepatomegaly and slide positivity rate was also higher (17.31%) in hepatomegalic individuals. It was lower (2.11%) in non-hepatomegalic individuals. The difference was statistically significant.

The sero-positivity rate was highest (94.23%) in individuals with hepatomegaly and lower (0.20%) in individuals without hepatomegaly. The difference was statistically significant.

TABLE 11

Slide positivity and sero-positivity according to Splenomegaly.

Splenomegaly	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Present	144	18	12.50	133	92.36
Absent	1376	22	1.60	11	0.80
Total	1520	40	2.63	144	9.47

(X^2=60.22, d.f.=1, P $<$ 0.001). (X^2=1278.3, d.f.=1, P $<$ 0.001)

FIG.-10

BAR DIAGRAM SHOWING S.P. R. & SEROPOSITIVITY RETE AMONGST INDIVIDUALS WITH AND WITHOUT SPLENOMEGALY

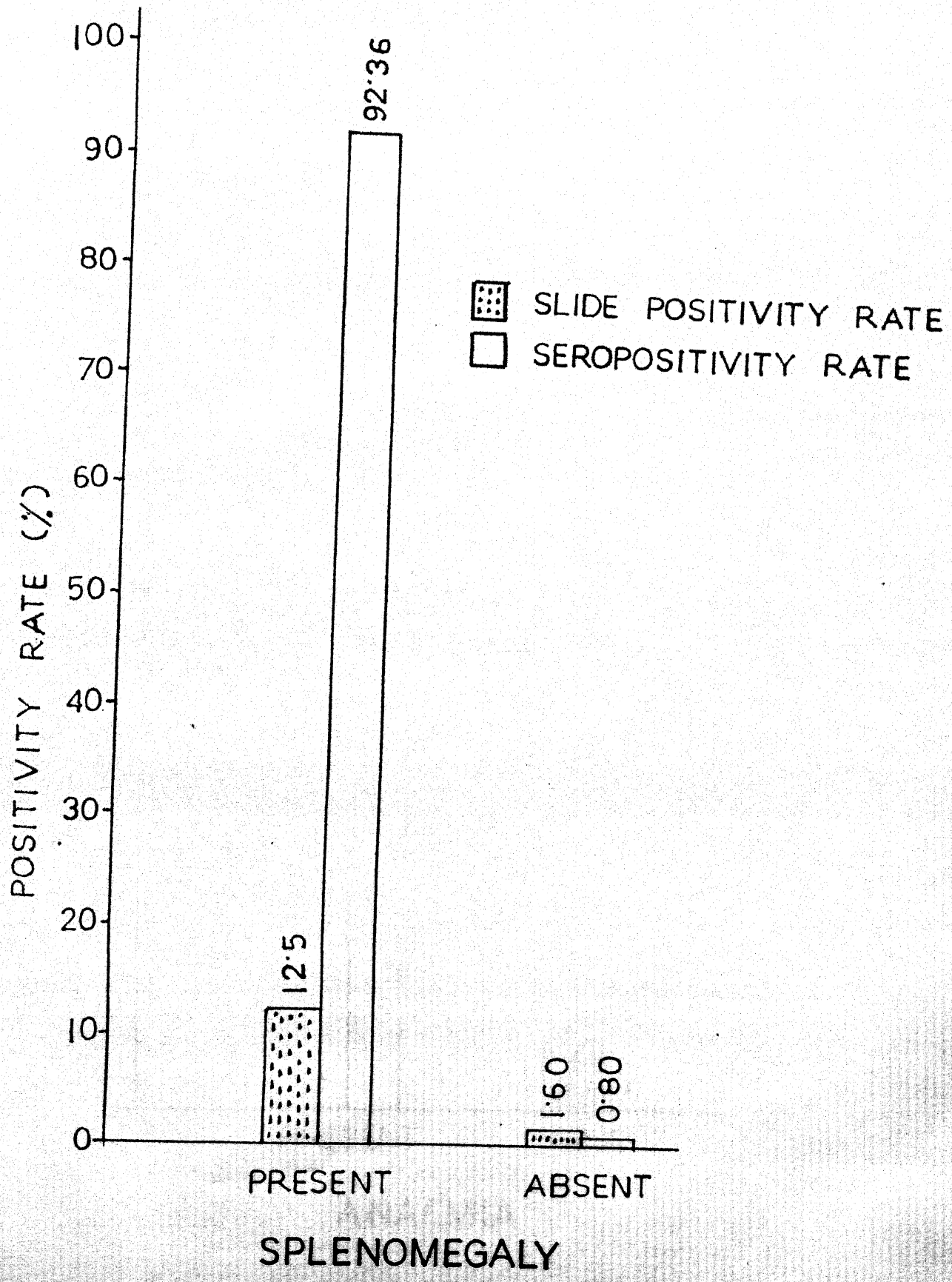
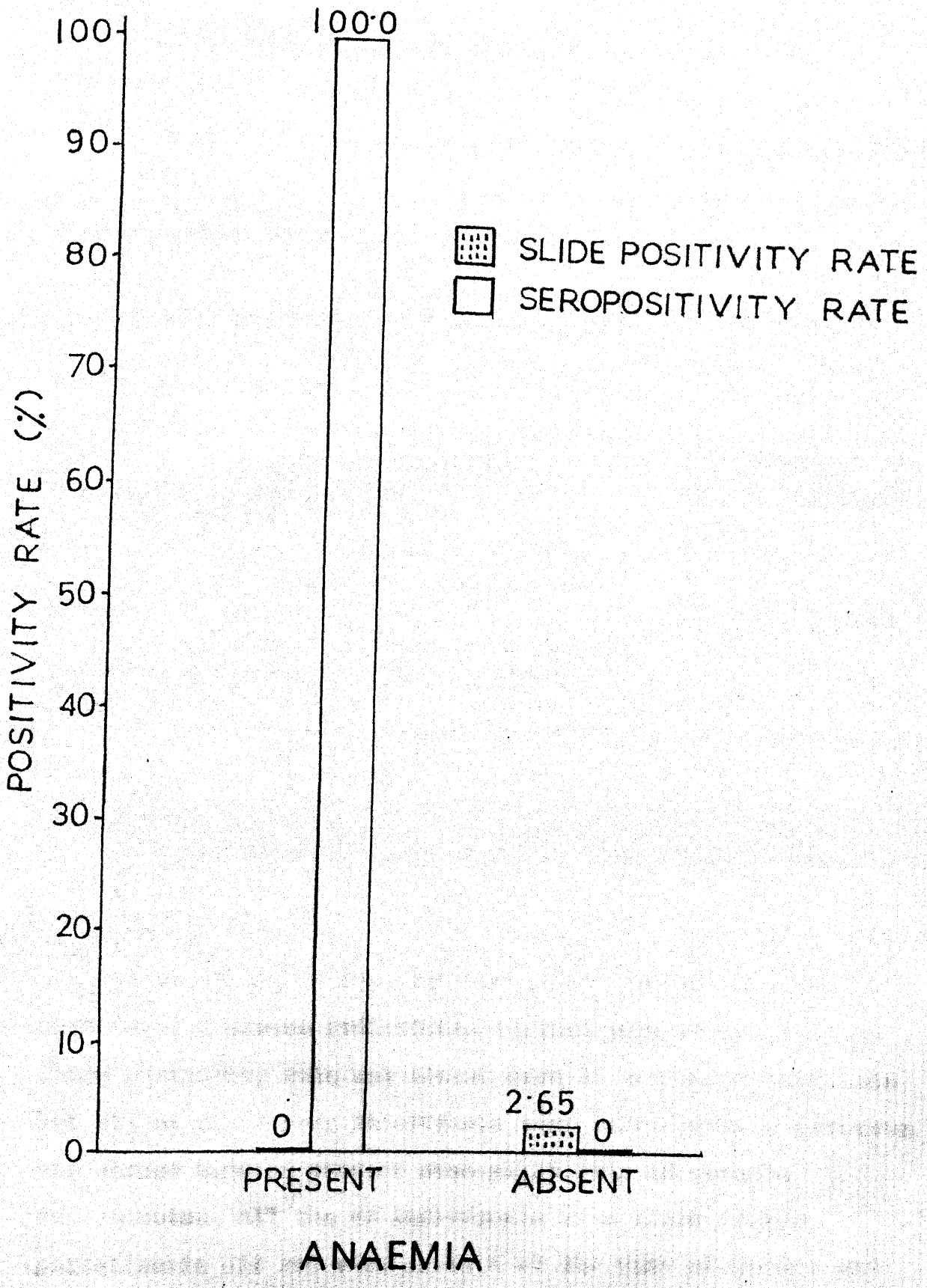


FIG.-11

BAR DIAGRAM SHOWING S.P.R. & SEROPOSITIVITY RATES AMONGST INDIVIDUALS WITH & WITHOUT ANAEMIA



3.2 Splenomegaly :

Table 11 and Fig. 10 shows that out of 1520 individuals, 9.47 percent presented with splenomegaly slide positivity rate was higher (12.50%) among individuals with splenomegaly and lower (1.60%) in individuals without splenomegaly. This difference was statistically significant.

TABLE 12

Slide positivity and sero-positivity according to anaemia.

Anaemia	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Present	11	-	-	11	100.00
Absent	1509	40	2.65	711	-
Total	1520	40	2.63	722	47.50

3.3 Anaemia :

Table 12 and Fig. 11 shows that out of 1520 individuals, eleven individuals (0.72%) were found anaemic. Slide positivity rate was almost zero in anaemic individuals but all of the eleven individuals were serologically positive and showed hundred percent sero-positivity in anaemic individuals. All the 40 individuals with slide proven parasitaemia did not show anaemia at the time of survey and 2.65 percent showed slide positivity in non-anaemic individuals.

FIG.- 12

BAR DIAGRAM SHOWING S.P.R. & SEROPOSITIVITY RATE AMONGST INDIVIDUALS WITH & WITHOUT RAISED TEMPERATURE

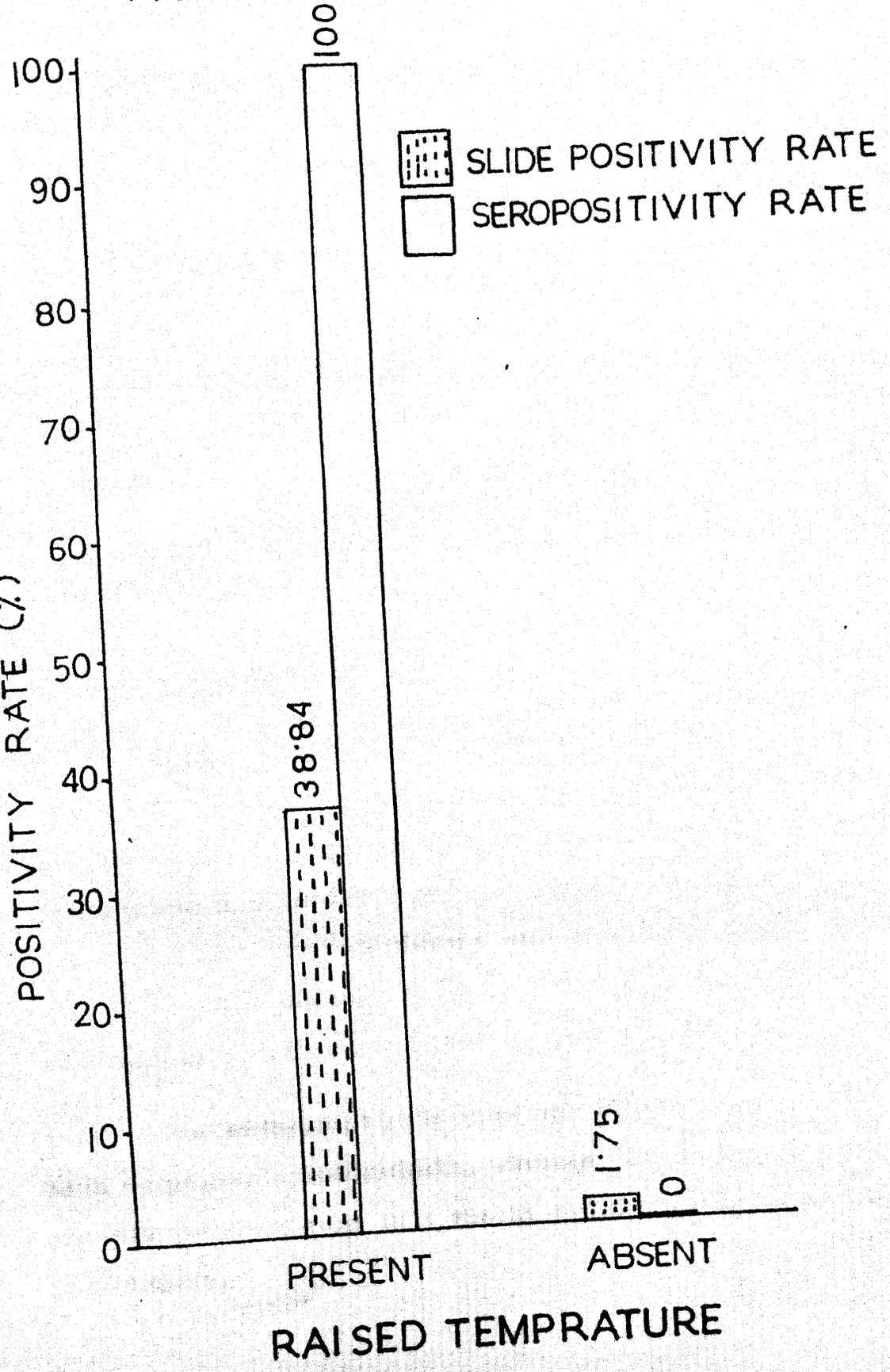


TABLE 13

Slide positivity and sero-positivity according to high temperature.

High temperature	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Present	38	14	36.84	38	100.00
Absent	1482	26	1.75	684	-
Total	1520	40	2.63	722	47.50

(X^2=173.567, d.f.= 1, P $<$ 0.001).

3.4 Raised Temperature :

Table 13 and Fig. 12 shows that the slide positivity and sero-positivity according to high temperature. In small number (2.5%) of individuals the history of high temperature was recorded during survey. The slide positivity rate in individuals with high temperature was higher (36.84%) whereas it was lower (1.75%) in afebrile individuals. This was statistically significant.

The sero-positivity rate was hundred percent in high temperature individuals, whereas high temperature was not observed even in a single serologically negative individuals.

FIG.-13

BAR DIAGRAM SHOWING S. P. R. AND SEROPOSITIVITY RATES AMONGST INDIVIDUELS WITH AND WITHOUT PAST HISTORY OF FEVER

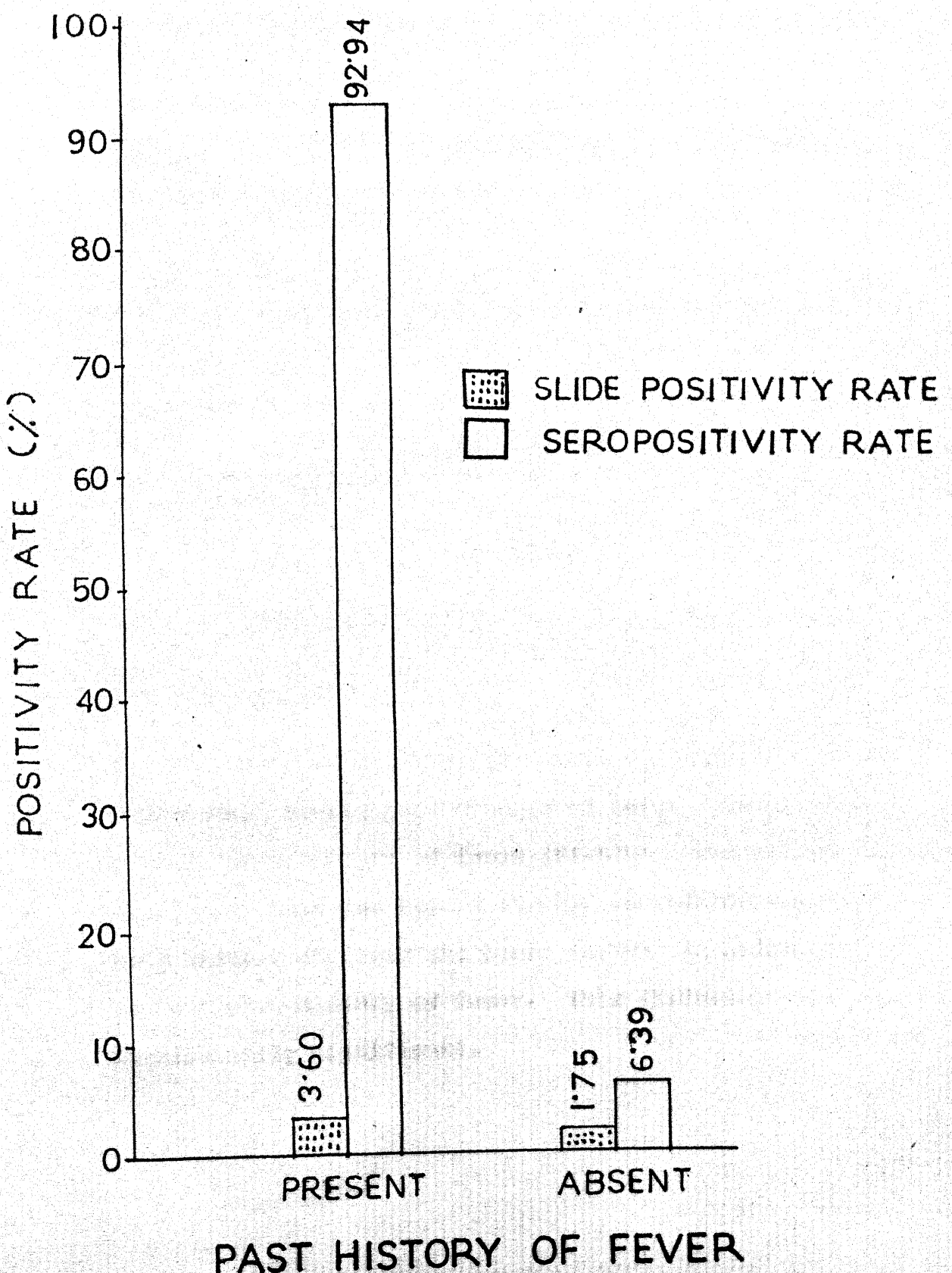


TABLE 14

Slide positivity and sero-positivity according to past history of fever.

Past history of fever	Total No. examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Present	722	26	3.60	671	92.94
Absent	698	14	1.75	51	6.39
Total	1520	40	2.63	722	47.50

(x^2=5.045, d.f.=1, P $<$ 0.10), (x^2=1338.06, d.f.=1, P $<$ 0.001)

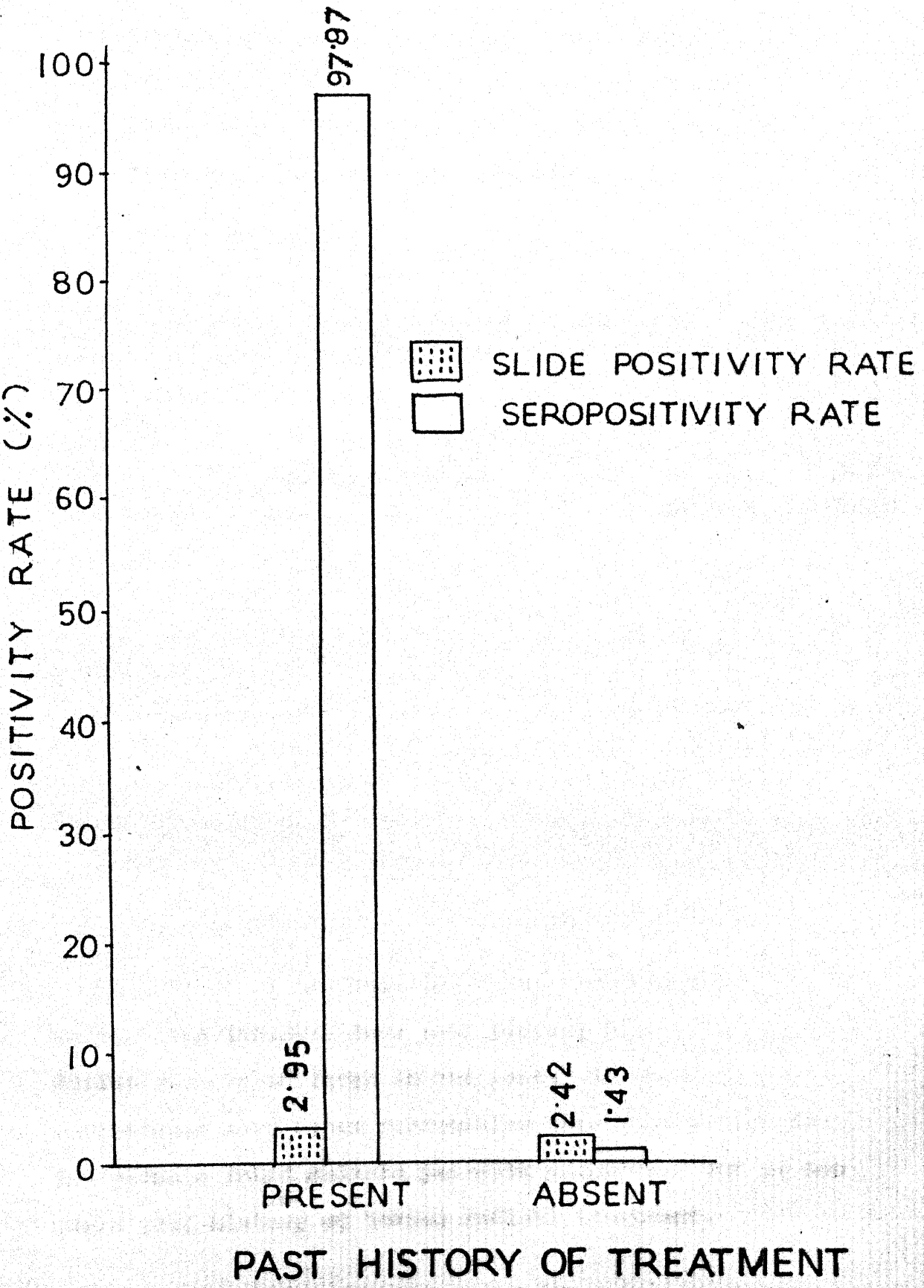
4. Past History :

4.1 Past history of fever :

Table 14 and Fig. 12 shows the distribution of individuals having past history of fever (47.50%) and without past history of fever (52.50%). The slide positivity rate was higher (3.60%) in individuals with past history of fever and lower (1.75%) in individuals without past history of fever. This difference was statistically significant.

FIG. -14

BAR DIAGRAM SHOWING S.P.R. AND SEROPOSITIVITY RATE AMONGST INDIVIDUALS WITH & WITHOUT PAST HISTORY OF TREATMENT



The sero-positivity rate was higher (92.94%) in individuals suffering from fever at the time of survey and have suffered in past and it was lower (6.16%) in individuals without past and present history of fever. This difference was statistically significant.

TABLE 15

Slide positivity and sero-positivity according to past history of treatment taken (presumptive/radical).

Past history of treatment Presumptive/ radical	No. of cases examined	Slide examination		Serological examination	
		No. found positive	Positivity rate(%)	No. found positive	Positivity rate(%)
Treatment taken	611	18	2.95	598	97.87
Treatment not taken	909	22	2.42	124	1.43
Total	1520	40	2.63	722	47.50

(X^2=0.385, d.f.=1, P $\gtrsim$0.75). (X^2=1413.0, d.f.=1, P$<$0.001)

4.2 Past history of treatment (Presumptive/radical):

Table 15 and Fig. 14 shows that the majority of individuals (40.20%) have past history of treatment taken during attacks of fever in the past. In this all the individuals have taken presumptive treatment except few individuals found malaria parasite positive. But no one could give history of taking radical treatment.

The slide positivity rate was higher (2.93%) in individuals with past history of treatment taken (presumptive) and lower (2.42%) in individuals without past history of treatment taken. This difference was statistically not significant.

The sero-positivity rate was higher (97.87%) in individuals with past history of treatment taken and lower (1.43%) in individuals without past history of treatment taken. This difference was statistically significant.

Correlation between slide positivity and sero-positivity :

Out of 1520 individuals examined, the forty individuals (2.63%) were detected positive for malaria parasite. Plasmodium vivax infection was observed in all positive individuals. During the same time, the sero-positivity was observed as 47.5 percent. In the forty individuals, all were also found positive serologically. Thus shows hundred percent sero-positivity in slide positive individuals. The sero-positive rate positively correlated with the slide positivity rate (Fig. 15).

FIG:- 15

CORRELATION BETWEEN SLIDE & SEROPOSITIVITY RATES

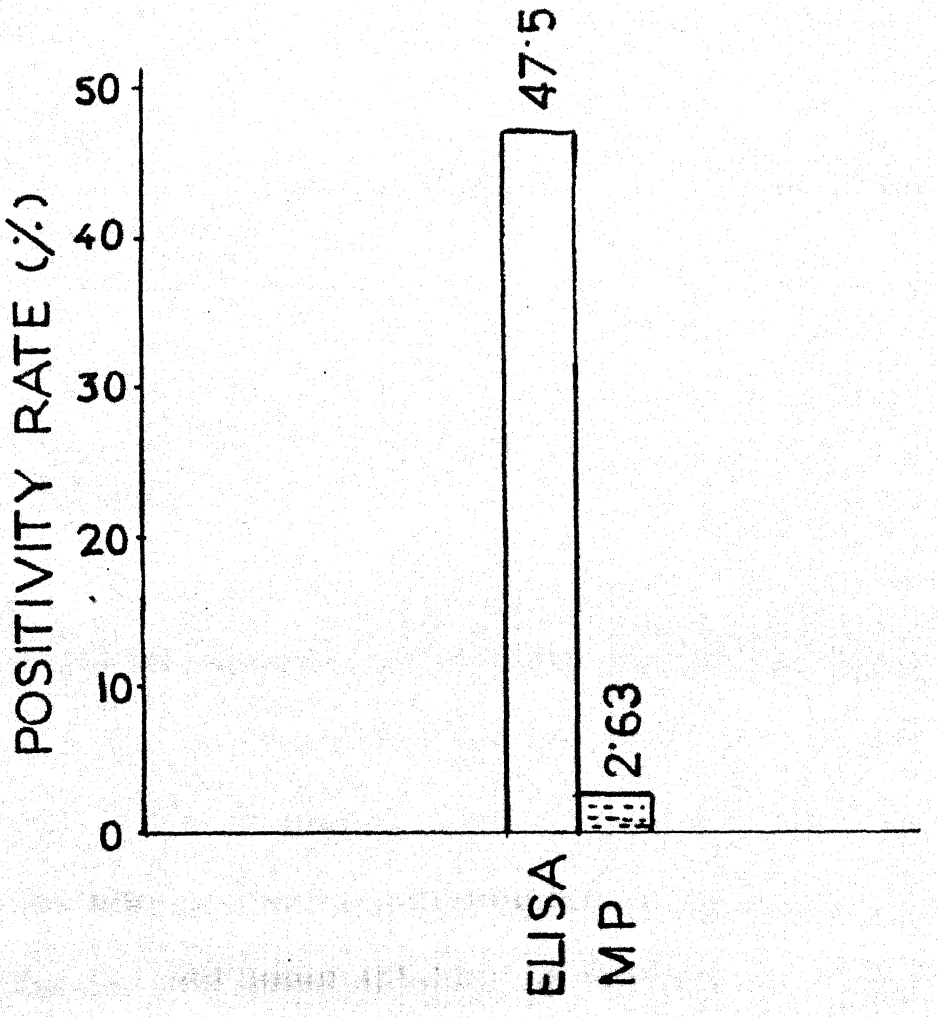


TABLE 16

Distribution of children (2 - 10 years) according to their sex.

Sex	Total No. examined	Slide examination (2-10 years)		Serological examination (2-10 years)	
		No.found positive	%	No.found positive	%
Male	189	3	1.59	25	13.23
Female	139	3	2.16	22	15.83
Total	329	6	1.83	47	14.33

5. Parasite rate :

Table 16 shows that the children between the ages 2 - 10 years showing malaria parasite in their blood films. The parasite rate was higher (2.16%) in female and lower (1.59%) in males. Total parasite rate was 1.83 percent. In the same age group sero-positivity was higher (15.83%) in females and lower (13.23%) in males.

TABLE 17

Distribution of ELISA value according to age.

Age	Total sera examined	ELISA value(E_{492} n.m.) optical density							
		0 - 0.2	0.2 - 0.4	0.4 - 0.6	0.6-0.8	0.8-1.0	1.0-1.2	1.2-1.4	Mean value
< 1	1	1							0.58
1 - 4	194	38 (36.54)	50 (48.08)	5 (4.81)	3 (2.88)	4 (3.85)	3 (2.88)	1 (0.96)	0.62
5 - 9	198	49 (24.75)	121 (61.11)	18 (9.09)	5 (2.53)	3 (1.52)	1 (0.50)	1 (0.50)	0.62
10 - 14	179	51 (28.49)	98 (54.75)	16 (8.94)	8 (4.47)	3 (1.68)	3 (1.68)	-	0.62
15 - 24	321	65 (20.25)	122 (38.01)	60 (18.69)	51 (15.89)	17 (5.30)	4 (1.25)	2 (0.62)	0.68
25 - 34	246	21 (8.54)	69 (28.05)	70 (28.46)	55 (22.36)	20 (8.13)	8 (3.25)	3 (1.22)	0.66
35 - 44	177	18 (10.17)	31 (17.51)	68 (38.40)	34 (19.21)	19 (10.73)	6 (3.39)	1 (0.56)	0.67
45 - 54	150	15 (10.00)	15 (10.00)	15 (10.00)	64 (42.66)	42 (28.00)	11 (7.33)	3 (2.00)	0.70
55 - 64	105	5 (4.76)	15 (14.29)	45 (42.86)	24 (22.86)	12 (11.43)	4 (3.81)	-	0.67
65 +	39	3 (7.69)	10 (25.64)	19 (48.72)	3 (7.69)	3 (7.69)	1 (2.56)	-	0.66
Total	1520	266 (17.50)	531 (34.93)	365 (24.01)	225 (14.81)	92 (6.05)	33 (2.17)	8 (0.53)	-

(Figures in parenthesis indicate percentage). Percentages are based on age-specific positive individual.

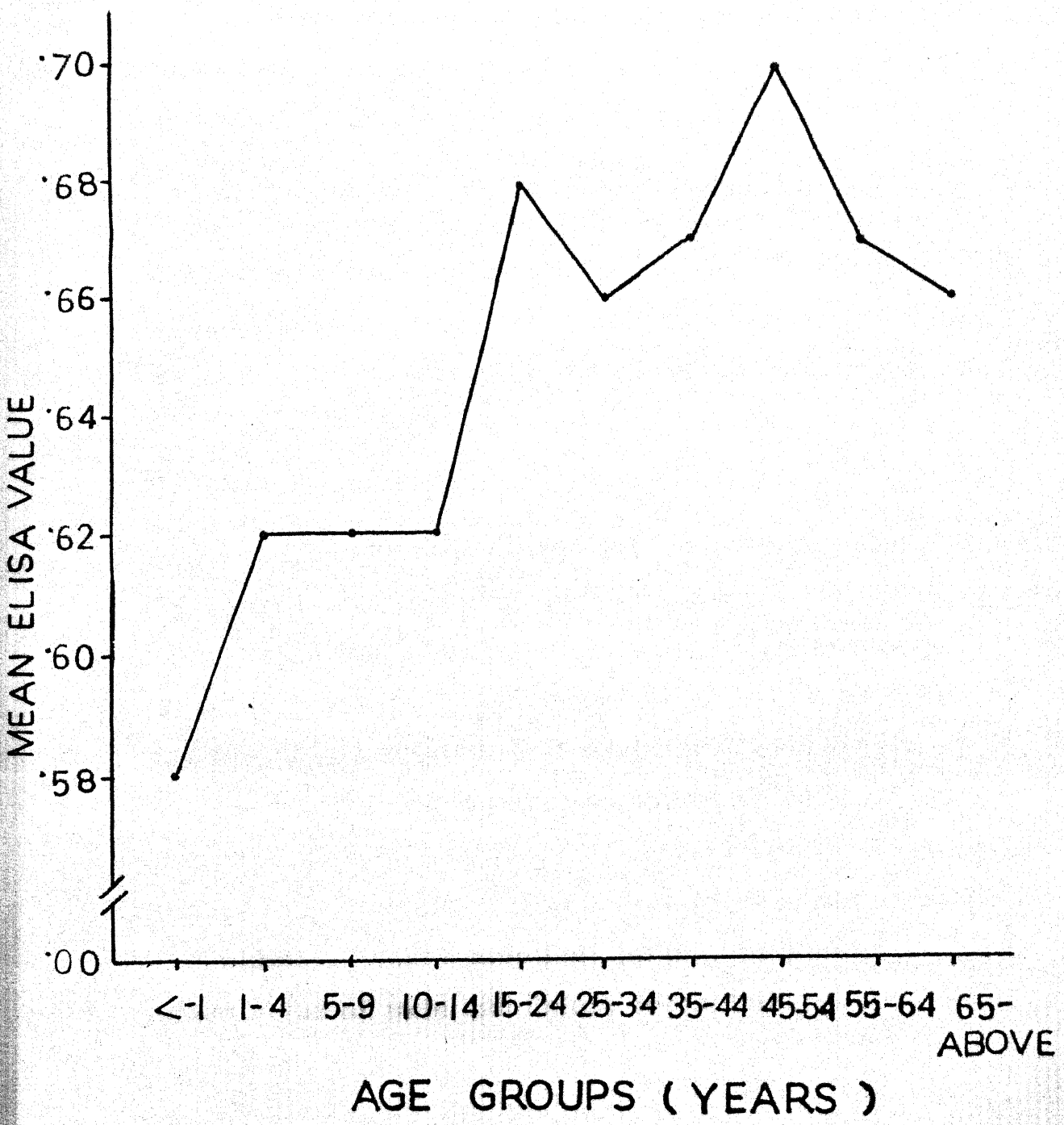
TABLE 18

Distribution of ELISA value by Sex.

Sex	No.of sera tested	ELISA value							Mean ELISA value
		0 - 0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.2	1.2-1.4	
Male	835	146 (17.48)	205 (24.25)	204 (24.43)	125 (14.97)	52 (6.23)	20 (2.39)	3 (0.36)	0.65
Female	685	119 (17.37)	246 (35.77)	161 (23.50)	100 (14.59)	40 (5.83)	14 (2.04)	5 (0.73)	0.64
Total	1520	265 (17.80)	531 (34.93)	365 (24.01)	225 (14.80)	92 (6.05)	34 (2.17)	8 (0.53)	

FIG.- 16

GRAPH SHOWING MEAN ELISA VALUE BY AGE



6. ELISA Test :

6.1 ELISA by Age :

Distribution of ELISA value according to age showed that the mean value of ELISA increased with the increase in age upto the age of about 54 years (Table 17, Figure 16). The mean values of ELISA showed a decreasing trend in the elderly age group i.e. over 55 years. The individuals showing the ELISA value of 7/ .40 E_{492} optical density with serum dilution 1 : 400 were taken as ELISA positive at this cut off point (Ray et al. 1983).

6.2 ELISA by Sex :

Table 18 shows distribution of ELISA value by sex. There were 28.36 percent male individuals who showed $\angle$ .40 O.D. (E_{492}) value and also 26.57 percent individual showed 7.40 O.D. (E_{492}) value. The mean ELISA value was 0.65 O.D. in case of male individuals. There were 24.01 percent female individuals who showed $\angle$ .4 O.D. (E_{492}) and also 21.05 percent individuals showed 7.40 O.D.(E_{492}) value. The mean ELISA value was 0.64 in case of female individuals. There were less difference seen in mean ELISA value of male and female individuals.

TABLE 19

Distribution of number of sera according to reaction grading at 32 dilution end point by age.

Age group	No.of sera	0		+		++		+++		++++	
		No.	%	No.	%	No.	%	No.	%	No.	%
< 1	-	-	-	-	-	-	-	-	-	-	-
1 - 4	20	2	10.00	5	25.00	9	45.00	4	20.00	-	-
5 - 9	30	4	13.33	12	40.00	11	36.66	6	20.00	-	-
10 - 14	37	9	24.32	11	29.73	13	35.14	3	8.11	1	2.70
15 - 24	134	20	14.93	30	22.39	54	40.30	28	20.90	2	1.49
25 - 34	158	28	17.72	28	17.72	60	37.97	42	26.58	-	-
35 - 44	126	31	24.60	18	22.22	50	39.68	27	21.43	-	-
45 - 54	106	20	18.87	23	21.70	38	35.85	24	22.64	1	0.94
55 - 64	79	12	15.19	17	21.52	25	44.33	24	30.38	1	1.27
65 +	29	6	20.69	6	20.69	14	48.28	3	10.34	-	-
Total	722	132	18.28	150	20.78	274	37.95	161	22.30	5	0.69

7. IIF Test Reaction :

7.1 IIF reaction by age :

Table 19 shows the reaction grading at 4+ with the test dilution giving a 2+ reaction used as the end point in ELISA positive individuals. Of the 722 ELISA positive individual tested with IIF, 132 (18.28%) have shown no reaction at 32 dilution end point or only a trace of fluorescence; 150 (20.78%) individuals showed a 1+ reaction which was only dimly fluorescent and very diffuse. There was 274 (37.95%) individuals samples have shown a 2+ fluorescent with the individual parasite quite diffuse, and 161 (22.23%) individual sample have shown a 3+ reaction which was brightly fluorescent but individual parasite was somewhat diffuse. There was 5 (0.69%) individuals who showed a 4+ brilliantly fluorescent with the individuals parasite sharp and easily brought to focus (Hall et al. 1978). By IIF test, the sero-positivity was observed as 60.94% in ELISA positive proven individuals at 1 : 32 dilution end point.

The higher (30.37%) reaction grading was also observed in age group 55 - 64 years, followed by 25 - 34 years age group. The lowest (8.11%) reaction grading was observed in age group 15.- 14 years.

TABLE 20

Distribution of number of sera according to reaction grading at 1:64 dilution end point by age.

Age group	No.of sera	0		+		++		+++		++++	
		No.	%	No.	%	No.	%	No.	%	No.	%
< 1	-	-	-	-	-	-	-	-	-	-	-
1 - 4	20	5	25.00	4	20.00	5	25.00	5	25.00	1	5.00
5 - 9	33	7	21.21	6	18.18	12	36.36	8	24.24	-	-
10 - 14	37	8	13.51	15	40.54	9	24.32	8	21.62	-	-
15 - 24	134	31	23.13	4	2.99	43	32.09	15	11.19	1	0.75
25 - 34	150	39	26.68	53	33.54	43	27.22	23	14.56	-	-
35 - 44	126	37	29.37	23	18.25	55	43.65	11	8.73	-	-
45 - 54	106	26	24.53	20	18.87	49	46.22	11	10.38	-	-
55 - 64	79	17	21.52	13	16.46	36	45.57	13	16.46	-	-
65 +	29	7	24.14	8	27.59	11	37.93	3	10.34	-	-
Total	722	174	24.10	186	25.76	263	36.43	97	13.43	2	0.28

Table 20 shows reaction grading at 1 : 64 dilution end point by age. There was 174 (24.10%) individuals who showed no reaction followed by a 1+ reaction in 186 (25.76%). 263 (36.43%) showed a 2+ reaction, 97 (13.43%) showed 3+ reaction and only 2 (0.28%) showed a 4+ reaction.

Out of 722 ELISA positive individuals, 372 (50.14%) showed a 2+ and above reaction. The positive reactivity in ELISA positive individuals was 50.14%. The higher (46.22%) positive reaction was observed in age group 45 - 54 years, followed by 55 - 64 years age groups and lower (24.32%) was observed in 10 - 14 year age group.

7.2 <u>IFP reaction by sex</u> :

Table 21 shows distribution of ELISA positive individuals according to their sex. Of the 404 males ELISA positive sera tested, there were 234 (57.92%) sera showed 2+ and above reaction grading at 1 : 32 dilution end point and 206 (42.08%) females. ELISA positive sera have also shown 2+ and above reaction at the same dilution end point.

TABLE 21

Distribution of number of sera according to reaction grading at 1 : 32 dilution by sex.

Sex	No.of sera	Reaction Gradings									
		0		+		++		+++		++++	
		No.	%	No.	%	No.	%	No.	%	No.	%
Male	484	71	17.57	79	19.55	144	35.64	87	35.65	3	0.74
Female	318	61	19.18	71	22.33	130	40.88	74	23.27	2	0.63
Total	722	132	18.28	150	20.78	274	37.50	161	22.30	5	0.63

TABLE 22

Distribution of number of sera according to reaction grading at 1 : 64 dilution by sex.

Sex	No.of sera	Reaction Grading									
		0		+		++		+++		++++	
		No.	%	No.	%	No.	%	No.	%	No.	%
Male	404	101	25.00	107	26.49	143	35.40	51	12.62	2	0.50
Female	318	73	22.96	79	24.84	120	29.70	46	14.47	-	-
Total	722	174	24.10	186	25.76	263	36.43	97	13.43	2	0.28

Table 22 depicts the reaction grading at 64 dilution end point by sex. There was 196 (49.51%) male ELISA positive sera which showed 2+ and above reaction grading, and 166 (52.20%) female sera showed 2+ and above reaction. At 1:32 dilution, out of 318 (33.4%) female sera, 206 showed 2+ and above reaction. There was 9.41 percent male and 12.56 percent female which dropped in 1 : 64 dilution end point.

TABLE 23

Indirect Immunofluorescence antibody titres in ELISA positive sera using antigen from strains of cultured plasmodium falciparum by age.

Age	No.of sera	IIF Antibody titre					
		256	128	64	32	16	8
< 1	-	-	-	-	-	-	-
1 - 4	20	-	-	11	13	-	-
5 - 9	33	-	-	20	17	-	-
10 - 14	37	-	-	17	17	-	-
15 - 24	134	-	-	59	86	-	-
25 - 34	150	-	-	66	102	-	-
35 - 44	126	-	-	66	77	-	-
45 - 54	106	-	-	60	63	-	-
55 - 64	79	-	-	49	50	-	-
65 +	39	-	-	14	17	-	-
Total	722			372	440		

(x^2=5.209, d.f.=8, P >0.750), (x^2=1.694, d.f.=8, P >0.97)

7.3 IIF Antibody titre by age :

Table 22 shows indirect immunofluorescence antibody titres in ELISA positive sera, using antigen from stains of cultured plasmodium falciparum by age. By using in-vitro culture, plasmodium falciparum stain antigen, antibody titre ranged from 1 : 8 to 1 : 256 dilution end point. In this test, starting dilution was 1 : 8 and significant titre were 1 : 32 and above by 95 confidence limit in relation to normals (Spencer et al. 1979; Mahajan et al. 1981). Therefore, an antibody level of 32 or more was considered as of sero-positivity. Amongst 722 ELISA positive individuals of all ages, 440 (60.94%) had positive titre (1 : 32) and 372 (51.52%) showed positive titre (1 : 64).

In relation to age, sero-positivity rate was increasingly higher (9.14%) in age group 25 - 34 year and lower (1.52%) in age group 1 - 4 years, and thereafter sero-positivity rate showed a decreasing trend upto age group 65+ (1.96%). The difference was statistically not significant by titre (1 : 32). The difference was also not significant by titre 1 : 64 in relation to age groups.

TABLE 24

Indirect Immunofluorescent antibody titres in ELISA positive sera using antigen from stains of cultured Plasmodium falciparum by sex.

Sex	No.of sera examined	IIF Antibody titre					
		256	128	64	32	16	8
Male	404	-	-	206 (51.00)	234 (57.92)	-	-
Female	318	-	-	166 (52.20)	206 (64.78)	-	-

7.4 IIF antibody titre by sex :

Table 24 shows, out of the four hundred four male individuals tested, 234 (57.92%) and 206 (51.00%) have shown positive antibody titre at 1 : 32 and 1 : 64 respectively. Twenty eight sera dropped at 1 : 64 antibody titre and forty dropped at 1 : 64 dilution in female individuals. Of the 318 ELISA positive female individuals, 206 (64.78%) and 166 (52.20%) had antibody titres of 1 : 32 and 1 : 64 respectively.

TABLE 25

Comparative results of slide positivity and sero-positivity by age.

Age group (year)	Total No.of sample examined	No. of Positive by							
		Slide examination		ELISA		IIF in ELISA positive			
		No.	%	No.	%	1:32	%	1:64	%
< 1	-	-	-	-	-	-	-	-	-
1 - 4	108	4	3.70	20	18.52	13	12.04	11	10.19
5 - 9	198	1	0.50	33	16.66	17	8.59	20	10.10
10 - 14	179	3	1.68	37	20.67	17	9.50	17	9.50
15 - 24	321	6	1.87	134	41.74	84	26.17	59	18.38
25 - 34	246	11	4.47	158	64.23	102	41.46	66	26.83
35 - 44	177	7	3.95	126	71.19	77	43.50	66	37.29
45 - 54	150	3	2.00	106	70.66	63	42.00	60	40.00
55 - 64	105	4	3.81	79	75.24	50	47.62	49	46.66
65 +	39	1	2.56	29	74.36	17	43.59	14	35.94
Total	1520	40	2.63	722	47.50	440	60.94	362	50.14

Note : No individual from < 1 year age group was positive by any test.

7.5 <u>Comparative results of slide examination/ELISA/IIF Test by age</u> :

Table 25 shows comparative results of slide positivity and sero-positivity by age. Of the 1520 sample examined, 40 (2.63%) samples were positive by slide examination and 722 (47.50%) were positive by ELISA test. Amongst the ELISA positive samples, 440 (60.94%) were positive by IIF test at 32 dilution end point, and 372 (50.14%) at 64 dilution end point.

TABLE 26

Comparative results of slide examination/ELISA/IIF Test by sex.

Sex	Total No.of Samples	No. of Positive by Slide examination No.	%	ELISA No.	%	IIF in ELISA positive 1 : 32 No.	%	1 : 64 No.	%
Male	835	21	2.51	404	48.38	234	57.92	206	50.99
Female	685	19	2.77	318	46.42	206	64.78	166	52.20
Total	1520	40	2.63	722	47.50	440	60.94	372	51.52

7.6 Comparative results of slide examination/ELISA/IIF Test by sex :

Table 26 shows comparative results of slide positivity and sero-positivity by sex. Slide positivity rate was higher (2.77%) in females and lower (2.51%) in males. The percentage of sero-positivity was higher amongst male (48.38%) and lower amongst females (46.42%). In ELISA positive samples, IIF antibody titre was higher in males (57.92%) and (50.99%) males at 1 : 32 and 1 : 64 dilution respectively, and was also higher in females (64.78%) individuals at 1 : 32 dilution and lower (52.20%) female individuals at 1 : 64 dilution end point.

TABLE 27

ELISA value and IIF reaction grading in slide positive individuals.

Sl. Code No.	ELISA value absorbance Unit	IIF reaction grading 1 : 32 dilution	IIF reaction grading 1 : 64 dilution	Sl. Code No.	ELISA value absorbance Unit	IIF Reaction Grading 1:32 dilu.	IIF Reaction Grading 1:64 dilution
30A	1.13	++	++	7 J	1.09	++	++
23 A	1.05	+++	+++	8 J	1.01	+	+
25 A	1.14	++	++	10 J	1.07	++	+
41 A	1.01	++	0	16 L	1.10	+++	++
50 A	1.05	++	+	17 L	1.12	+++	+++
52 A	1.16	++	+++	18 L	1.05	+++	++
56 A	1.46	+++	+++	19 L	1.13	+++	+++
87 A	1.00	++	++	21 L	1.33	++	++
25 B	1.15	+++	+++	22 L	1.23	++	++
20 B	1.00	+	0	23 L	0.99	+	+
22 B	1.13	+++	+++	24 L	1.09	++	++
30 B	1.10	+++	+++	26 L	1.50	+++	++
35 B	1.21	++	++	32 M	0.94	++	++
36 B	1.35	++	++	3 P	1.00	++	+
79 F	1.01	+++	+++	4 P	1.02	++	+
22 N	1.20	++	++	10 P	1.16	+++	+++
23 N	1.19	++	++	11 P	1.15	+++	+++
16 I	1.00	++	+	12 P	1.09	+++	+++
39 I	1.03	++	++	13 P	1.03	+++	++
93 I	1.24	++	++	17 P	1.10	+++	++

8. Sero-positivity in slide positive cases :

Table 27 shows ELISA value and IIF reaction grading in slide positive individuals. Of the 40 individuals who were slide positive, all the forty were ELISA positive as well. They have shown ELISA value ranging from 0.99 to 1.46 optical density at 492 n.m. and in all of them, three to four fold rise of ELISA value (more than 0.39 optical density at 492 n.m. ELISA value) could be demonstrated. Of the forty confirmed malaria positive individuals, 36 showed IIF test positive reaction at 1 : 32 dilution end point and 31 showed positive reaction (2+ or more) at 1 : 64 dilution end point.

TABLE 28

Validity of ELISA test at above 39 absorbance unit.

ELISA results	Slide results		Total
	Positive	Negative	
Positive	40	682	722
Negative	-	798	798
Total	40	1480	1520

Sensitivity = 100% ; Specificity = 53.92%.

TABLE 29

Validity of IIF test (a) at cut off titre of 32

IIF results	Slide results		Total
	Positive	Negative	
Positive	36	404	440
Negative	4	278	282
Total	40	682	722

Sensitivity = 90.00% ; Specificity = 40.76%.

TABLE 30

Validity of IIF test (b) at cut off titre of 64.

IIF results	Slide results		Total
	Positive	Negative	
Positive	31	341	372
Negative	9	341	350
Total	40	682	722

Sensitivity = 77.50 ; Specificity = 50.00%.

9. Validity of ELISA test :

Table 28 shows the specificity and sensitivity of the test was calculated at the 0.39 optical density (E_{492}) as cut off point. It was found that specificity of the test in low transmission study area, was 53.92%. Test was very sensitive at the 7 .40 optical density (E_{492}) cut off point and showed 100 percent sensitivity in slide positive individuals.

10. Validity of IIF test :

Table 29 & 30 shows during transmission season in the area, the specificity of the test at cut off titre of 1 : 32 was 40.76%. at 1 : 64 it was 50.00 percent. The sensitivity of the test at the cut off titre of 1 : 32 was 90.00 percent, at 1 : 64 it was 77.50 percent. Whereas at cut off titre of 1 : 32 though the sensitivity was good (90.00%), the specificity was lower (40.76%). At cut off titre of 1 : 64 though the specificity was slightly better but the sensitivity was poor (77.50%).

Hence, a cut off titre of 1 : 32 appeared to be best suited for studying the sero-epidemiology and was used in the study.

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DISCUSSION

DISCUSSION

Malaria continues to be an important health problem in most of the countries of South East Asia Region and requires a flexible epidemiological approach with available resources. However, the success in integrated malaria control could be seriously impeded without a sound knowledge of local epidemiology of malaria. Since during past three decades, socio-economic status as well as the habitats and eco-systems of malaria vectors and parasite, deforestation, flooding, irrigation, the green revolution, vectorial efficiency of anophilines and their sibling species and uncontrolled population movements have also had a significant impact on malaria epidemiology (T.R.S. 547).

The serology of malaria has a long and varied history. Only in the last two decade, new serologic techniques such as the indirect fluorescent antibody test (IFF) and Enzyme linked immunosorbent assay (ELISA) have been explored for use in solving problems associated with epidemiology, specialise and diagnosis.

1. Population under study :

The study under discussion was conducted in rural area of Chirgaon, Jhansi (U.P.). A population survey for

slide and seropositivity for malaria was carried out in the area. The studied population consisted of 1520 individuals from 269 families out of 290 families. An attempt was made to include all ages and both sexes in the study to know the serological profile of the population. The study area was known hypo-endemic (API 2.6) for malaria. Hence it was decided to have 20 percent sampling of the population to look for correlation between serological and slide positivity.

Lobel et al (1976) in sero-epidemiological investigation of malaria in Guyana, where malaria control was in maintenance phase included 20 percent of the estimated population of survey sectors. Mantani et al (1979) took a small sample. They examined only 60 and 50 households in first and second surveys. The total population of surveyed area was 39,033.

The survey was done during September-October 1987, since it was peak of transmission season. Srivastava et al (1975) studied in the same district and showed, August, September and October were the favourable months for the transmission of both the species viz. Plasmodium vivax and Plasmodium falciparum, with September and October the peak months. It was due to increased vector mosquito density.

1.1 Male Female ratio :

The male and female ratio of sample in our study was 1000 : 818 as compared to 1000 : 845 for the population of area (Census, 1981). The male female ratio was less as compared to Census 1981. The young adults male and females contributed 30.92% and 31.37% respectively. The paediatric population sampled in the study was 31.72 percent. Male accounted for 18.03 percent and female 13.69 percent. However, only 1 (0.07%) individual was in age group $\leq$ 1 year sampled during survey. This disparity was due to non co-operation of parents of children and adults were not available during day time and due to unavoidable circumstances, could not be contacted in the evening in that particular village.

There was poor co-operation from adult females due to practice of purdha. They refused to come out from the house for interrogation and investigations. But this was not expected to effect the results as sex differences do not produce variation in serological studies for malaria (Lobel et al. 1976).

2. Bio-social Characteristics of Population :

2.1 Age :-

The present study revealed that the slide positivity rate (SPR) for malaria varied with age. The higher (1.68%) slide positivity rate was observed amongst

individuals of 25 years and above. It was found lower (0.50%) amongst individuals belonging to age group 5-9 years. Slide positivity was 3.85 percent in the 1-4 years age group which indicates that fresh transmission is occuring in this area. No comment on age group < 1 year could be made due to non-availability of samples from this age group. There was no significant ($P > 0.25$) statistical difference in slide positivity rates between age group 1-4 years and 15-44 years.

Choudhary et al (1984) carried out a study and classified the population on the basis of the clinical history of malaria observed that all age groups were affected by the disease but that there was a progressive increase of malaria attacks to 16-25 years of age, where the rates reached the maximum level.

Pattanayak et al (1978), while attempting to analyse the dynamics of re-establishment of malaria endemicity, found that the malaria incidence in the age group 9-14 years was quite high even at initial stages of malaria epidemics in comparison to other age groups. The prevalence of malaria among different age groups is subject to wide variations. According to Macdonald (1957) in the zone of epidemic malaria, there had been a trend for malaria prevalence to remain more or less evenly distributed among different age groups during malaria epidemics. The value of malaria indices among children and adults were

used extensively for identification and specification of endemicity in malarious area (Pampana, 1969). The dynamics of these indices in areas subject to anti-malarial measures served as a basis for assessment of these measures (Bruce-Chwatt, 1985).

Verma et al (1983) observed overall slide positivity rate of 3.42%, without any significant difference for various age groups. This corroborates well with our study.

The higher (7.8%) slide positivity was observed by Mamtani et al (1982). Uprety et al (1982) observed 6.8% slide positivity rate in afebrile healthy children of 2-9 years of age, although the positivity was higher (45.5%) in febrile individuals.

In our study, the sero-positivity rate of 19.23 percent was seen in 1-4 years age group and higher (75.24%) in 55-64 years & above age groups. Age has been an convenient variable in interpreting the results of serological technique. This can tell whether transmission occuring is fresh or it is previous experience of malaria. All age groups are expected to be involved where fresh transmission of malaria is occuring. The immunity to malaria rises as the age of individual increases with consequent reduction in parasite rate (Dasgupta et al, 1965). The N.I.C.D. has conducted serological studies in erstwhile hypo-endemic malarious areas of the country

during 1970s, and it was observed that the population below 5 years of age had hardly any malarial experience. It was only higher age group who showed high titres.

This correlated very well with our observations that the sero-positivity was significantly low in the age group 1-14 years when compared with 15-44 years and 45-64 years age group ($P < 0.001$).

The overall sero-positivity was 45.7 percent. Out of the total ELISA positive individuals, 60.94% individuals were positive at 1:32 dilution end point by indirect immunofluorescence (IIF) test and 50.14 percent at 1:64 dilution were also positive by IIF test.

In the literature, the data on comparative studies of ELISA and IIF are extremely scarce. our findings are in accordance with Collins et al (1972) who observed percentage positive to P.falciparum antigen ranging from 47.2% to 86.0% and for Plasmodium falciparum or P. malariae antigen percentage positive ranging from 60.0 to 80.0% and for P. malariae from 52.0 to 80.4%.

Collins et al (1972) also reported 78.4 percent sero-positivity for younger groups and 92.8 percent for older groups. It is apparent that in younger age group the IIF test failed to detect a number of those with patent parasitaemia.

In our study, 100 percent of malaria cases could be detected by a P.falciparum ELISA test and 90.00 percent at 1:32 dilution and 77.50% at 1:64 dilution by IIF test. This corroborates well with Srivastava et al (1983) who observed 100 percent sero-positivity of malaria patients (Group I), 28.78 percent of patients varied origin (Group II), 98.75 percent of random hospital patients (Group III) and 15.48 percent of the normal healthy subjects (Group IV). Ray et al (1983) obtained 85.1 percent sero-positivity in malaria cases by a P.falciparum ELISA test. Agarwal et al (1981) obtained 90.7 percent sero-positivity by using P.cynomulgi antigen and 79.9 percent sero-positivity rate by IIF test, using P. knowlesi antigen.

2.2 Sex :-

Female dominated the scene in present study as slide positivity was higher (2.77%) than males (2.51%). The difference was not significant (P >0.75). On the contrary, Srivastava et al (1975) observed higher slide positivity rate for males (4.60%) as compared to females (2.29%). Males sleep outdoor more than females, thus resulting in a frequent man-mosquitoes contact.

Baljeev et al (1986) in Mayurbhanj District (Orissa), did not observed significant difference in slide positivity rates in males (12.5%) and females (10.6%).

It was not clear whether it was due to higher exposure of adults males to malaria or due to influence of local socio-economic status, ethnic groups, or the attitude of parents especially mothers towards male and female children regarding treatment and ignorance about availability of free services in the village. There was no relationship of sex to species of malaria parasites and no significant difference between infection rates of males and females with any species of parasite (Sweet, 1933).

Sero-positivity rate was higher in males than female and the difference was statistically significant ($p < 0.25$). Similar observations were made by Collins et al (1971) in a study carried out in Ethiopia, found seropositivity 36.7 percent and 4.3 percent at low and high attitude respectively. The seropositivity was higher among males than females.

2.3 Religion & Caste :-

In our study, majority (48.60%) belonged to backward caste, followed by scheduled caste (35.72%), upper caste (14.8%) and Muslims (0.86%). However, the slide positivity rate was highest (7.69%) for muslims, whereas for scheduled and backwards it was found to be 3.68 and 1.89 percent respectively. This difference was not significant ($p > 0.10$).

Srivastava et al (1975), in the same district found more cases amongst Hindus which largely reflect the population composition during recent years.

2.4 Marital Status :-

The present study revealed high (3.19%) slide positivity rate in married and lower (1.93%) in unmarried. The difference was significant ($P < 0.01$). The sero-positivity rate was highest (65.80%) in widow/widower/ divorcee, followed by (62.22%) in married and lowest (22.77%) in unmarried individuals. The difference was significant($P < 0.001$). It is due to the fact that married individuals belong to higher age group.

2.5 Literacy Status :-

Our study revealed that malaria is more common amongst illiterates. Slide positivity rates amongst illiterates and literates were 4.29 percent and 2.35 percent respectively, which was statistically significant ($P < 0.025$). On the contrary, Verma et al (1983) did not observed any significant association of slide positivity rate with literacy status.

The sero-positivity was 58.78 percent in illiterate and 34.00 percent in literate. It was found that sero-positivity rate declined with increasing literacy status.

2.6 Occupation :-

Our study revealed a significant ($P < 0.025$) association between slide positivity of malaria and various occupations. Association between sero-positivity and various occupations was also significant ($P < 0.001$). The slide positivity rate was higher (11.11%) in individuals engaged in service in thermal power project, railways etc. and declined amongst farmers (2.94%), housewives (3.19%), followed by labour (2.04%). It was lower amongst student (1.85%) and children (1.66%).

In India, the majority of total annual malaria cases occur among various categories of Agricultural labour (Pattanayak, 1981). The rest of cases occur in urban and other areas of the country (Sharma, 1984; Kondrashin & Dixit, 1985). The risk to acquire malaria is higher among mobile workers and among those exposed to mosquito bites in open air on account of their occupational requirements (Kondrashin, 1986). SPR and slide falciparum rate (SFR) in particular, was higher in labour force engaged in tea plantations, in forest economy bamboo cutting in jungles, as compared with same index among local inhabitants of neighbouring area (National Malaria Eradication Programme, 1984). Construction workers at development projects, fishermen, coal miners and labour employed in number of thermal power projects

as well as railways in the peripheral part of the country show a relatively new pattern of labour movement and had shown higher SPR and SFR. There was explosive malaria situation with evidence of chloroquine resistant P. falciparum (Ray, 1984; Raj Gopalan, 1984; Panicker et al. 1984; Panicker & Raj Gopalan, 1986).

Comparative sero-epidemiological studies among migrant workers and the sedentary population residing around Sathanam Reservoir in Tamil Nadu revealed that the former had a higher positivity rate as compared with the latter (Hyma & Ramesh, 1988).

2.7 Social Class :-

Our study revealed a higher (4.38%) slide positivity rate amongst individuals from Social Class V to those from Social Class IV (2.02%) and Social Class III (2.61%). No individual was found positive amongst Social Class I and Social Class II. The difference was significant ($P < 0.05$). It was due to low socio-economic status, individuals were living in ill-ventillated, ill-lighted and unhygienic houses surrounded by various types of water collections. Poor people usually live in huts/ kutcha houses and keep cattle inside their residences, thus resulting in mosquito nestling places with them. Verma et al (1983) has reported higher SPR (3.61%) for those belonging to social class V as against about 2% for Social Class IV.

The sero-positivity rate was higher (54.48%) in Social Class V, followed by Social Class IV (46.10%) and Social Class II (40.74%). Difference was significant (P $\angle$ 0.010). Malaria, though common in all groups of society, has been significantly increasing among economically backward classes, inhabiting areas with difficult accessibility on the periphery, and where malaria eradication was never achieved (Ray, 1979; Kondrashin, 1983).

2.8 Over-crowding :-

Slide positivity rates observed by us were 2.93 percent and 2.22 percent in individuals residing in over-crowded and uncrowded conditions respectively. The difference was not significant (P $\angle$ 0.25). The sero-positivity rate for individuals residing in crowded and uncrowded conditions were 49.99% and 45.41% respectively. This difference was insignificant (P $\angle$ 0.01).

Malaria is an exclusively local phenomenon governed by the presence of parasite vector and suitable environmental conditions in the community. Its distribution varies from village to village, and town to town and even from ward to ward in the same community depending on malariogenic conditions. Kondrashin & Orlov (1985) observed positive correlation between P.vivax incidence and population density as such the most intensive foci of P. vivax are situated usually in over-populated plain areas.

3. Slide positivity and seropositivity in relation with Clinical Manifestations :

3.1 Hepatomegaly :-

In our study, slide positivity rate was higher (17.31%) in hepatomegalic individuals and lower (2.11%) in individuals without hepatomegaly. Hepatomegaly was significant in slide positive individuals ($P < 0.001$). The sero-positivity rate was higher (94.23%) in individuals with hepatomegaly and lower (0.20%) in individual without hepatomegaly. The difference observed was significant ($P < 0.001$). Dasovitz & J.J. Saave (1965) in a study of immunity to malaria in protected and unprotected groups showed the liver enlargement rates, for all age group were lower than spleen rate but with the advance of age there was a decrease in liver enlargement rates. Liver rates were strikingly decreased of the protected population in contrast to unprotected population.

In congenital malaria, hepatomegaly and jaundice with haemolytic anaemia is common in an infant. The diagnosis is confirmed only by detection of the malarial parasite in the peripheral blood of the infants (Thompson et al. 1976).

3.2 Splenomegaly :-

Out of 1520 individuals, 144 (9.47%) had splenomegaly and showed 12.5 percent slide positivity.

Further analysis of these 164 individuals with splenomegaly 92.36 percent showed sero-positivity and rest were sero negative. Slide positivity was 1.60 percent in individuals without splenomegaly. The difference was statistically significant ($P < 0.001$).

Thomas et al (1981) conducted a sero-epidemiological study on aborigine children in Orang Asali Malaysia, revealed that the falciparum antibody prevalence rate was 84.6% as against to spleen rate (81.8%) and parasite rate (43.3%). There was positive correlation between sero-epidemiological study and spleen rate, particularly in the age group upto 9 years old. Splenomegaly is a good clinical manifestation for diagnosis of malaria during epidemics and in hyper-endemic areas as it gives on the spot results but it is of no value in low endemic areas where it does not depict the true prevalence, nor it is useful in monitoring the progress of malaria control programme. All patients with splenomegaly do not have malaria and all patients of malaria do not have splenomegaly. In view of the fact that the population in this rural community do take prompt presumptive treatment which prevents spleen from becoming enlarged, and that there is negligible difference in individuals with and without parasitaemia. However, higher sero-positivity rate was found in individuals with splenomegaly, consequent upon a sustained malaria challenge.

It was unlikely that malaria was the aetiological factor in the splenomegaly of these individuals. These results therefore confirm that spleen enlargement is an unreliable method for epidemiological assessment of malaria when, as at present, widespread use of anti-malarials is prevalent. Vander Kaay also obtained similar results in an epidemiological study carried out in Surinam in 1973-74. Mamtani et al (1979) also obtained similar results in a serological survey for malaria in a rural community of Delhi.

3.3 Anaemia :-

In the study, slide positivity rate was more in anaemic individuals, but all the individuals were serologically positive. This is due to the fact that they have suffered from malaria in the past and there may be other causes of anaemia in the population studied. All of the 40 individuals with slide proven parasitaemia did not show anaemia at the time of survey. It was clear from the results of slide positivity that anaemia is not a constant manifestation in recent infections, whereas, 2.65 percent slide positivity in non-anaemic individuals indicate that anaemia is common in chronic malarial infections as a remote manifestation.

4. Past History :

4.1 Past history of fever :-

There were 47.50% individuals with past history of fever and 52.50% individual without past history of fever. The slide positivity rate was higher (3.60%) in individuals with past history of fever and lower (1.75%) in individuals without past history of fever. The difference was statistically significant ($P < 0.01$). The sero-positivity rate was higher (92.94%) in individuals with past history of fever and it was lower (6.16%) in individuals without such history. The difference was statistically significant ($P < 0.001$).

Fever was classified as typical when it was intermittent and associated with chills and rigors; and atypical when it was either continuous or remittent without chills and rigors. Sharma et al (1985) observed body temperature in malaria patients ranging from 37.3°C to 41°C. Fever was more acute in Falciparum than Vivax malaria.

It would therefore be safe to conclude that fever remains an important and reliable symptom of malaria. Sehgal et al observed the same findings in Thailand. Nantani et al (1979) also observed the same phenomenon in the rural community of Delhi and suggested that malaria can occur in a typical form more often than the physicians might believe.

4.2 Past history of treatment (Presumptive/Radical) :-

The slide positivity rate was higher (2.95%) in individuals with past history of presumptive treatment and lower (2.42%) in individuals without past history of treatment. The difference was not significant ($P > 0.75$). The sero-positivity rate was higher (97.67%) in individuals with past history of treatment taken and lower (1.43%) in individuals without past history of treatment taken. The difference was statistically significant ($P < 0.001$). Serology in general, confirmed and extended results of slide examination and it was successful where slide examination failed, in detecting persons with malaria contact and possibly with sub-patent parasitaemia. Some of these reactions may have represented residual antibody from cured infections. Following anti-malarial treatment antibody titre declined. Schizonticidal drugs or natural immune responses of individuals may decrease antibody titres from significant levels. Such infections in individuals may exist for almost 1 year but this is only a minimal time (Hornstein et al, 1965).

5. Parasite rate :

In the study, the overall parasite rate was 1.83 percent. It was higher (2.16%) in females and lower (1.59%) in males. In the age group 2-10 years, the higher sero-positivity (13.69%) was observed in males as compared to females (13.23%). Similar finding was reported by

Mantani et al (1979) in a rural community of Delhi, who observed parasite rate 3.22 percent. Verma et al (1983) revealed an overall parasite rate of 3.42 percent which was higher (4.60%) for males and lower (2.29%) for females. The difference was not significant. On account of quite scanty work in recent years on the type of subject considered, the results of present study are difficult to be interpreted widely. However, in a study in meseendemic area, Upraty et al (1982) obtained 6.8% slide positivity rate in afebrile healthy children of 2-9 years, although the positivity was higher (45.5%) in febrile ones. Slide examination can only indicate the presence and absence of patent parasitaemia at the time of examination. It does not indicate individual malaria experience (Kagan, 1972). Absence of patent parasitaemia can be misleading since patency is influenced by immune status of individuals and use of anti-malarials.

A survey carried out in the Gambia (Harverson, Wilson & Hall, 1968) showed good concordance between the parasite rate and F.A. tests in the rural area when the transmission was at high level. Voller et al (1980) conducted a study in West Africa Savanna , using ELISA technique, found that many young infants were ELISA negative even though they had previous proven parasitaemia. This may be due to these young infants are not sufficiently mature to mount an effective humoral response and immune-depression might play a part.

6. ELISA Test :

6.1 Validity of ELISA test :-

Enzyme linked immunosorbent assay has been applied in the diagnosis of many infectious diseases (Voller et al. 1976). The present study reports evaluation of micro-ELISA in malaria and confirms the finding of Ray et al (1983). That in vitro cultured P. falciparum is an excellent source of antigen for this test to detect antibodies. Between the three antigen batches the P. falciparum strain had undergone over 100 more sub-cultures. Parasite did not show any changes so far as antigenicity is concerned with regard to micro-ELISA test. It was also obvious that with higher parasitaemia of the culture, the yield of antigen was more. The data on replicate testing of the test and reference sera within the same batch as well as with three different batches of antigens showed consistent results indicating the usefulness of the test. In this report, at a serum dilution of 1:400, 93.4 percent of normal individuals showed reaction upto 0.4 whereas the rest showed reading between 0.4 - 0.6 O.D. In contrast, Spencer et al labelled a reading of 0.3 at dilution of 1:40 as negative and no positive reaction was noted amongst normal individuals at 1:80. In a study on a normal healthy Indian population (Nahajan et al. 1981), using Antus P. Falciparum antigen, non-specificity was found to be 6.06 percent which is comparable to our results. However,

in this study the results were read visually 1 : 100 was considered as the cut off point.

In our study, at a serum dilution of 1 : 400, 32.50 percent of individuals showed reaction upto 0 - 0.4 O.D.(E_{492}), 24.01 percent showed 0.4 - 0.6 O.D. at (E_{492}) reaction, 14.01 percent individual showed 0.6 - 0.8 O.D. (E_{492}), 6.05 percent showed 0.8 - 1.0 O.D. (E_{492}) and rest other showed upto 1.4 O.D. (E_{492}). All the slide positive individual showed more than 1.0, O.D. (E_{492}) reaction.

The sensitivity and specificity were calculated at cut off point (7 .40). The 100 percent sensitivity of the test in individuals with patent P.vivax parasitaemia reported here confirmed earlier studies. There was no difference in sero-positivity in case of first attack and more than one attack showing that this test can detect very early antibodies (Mahajan et al, 1981). Ray et al (1983) also observed 100 percent sero-positivity in all the 11 cases of P.falciparum infection from Haryana State. Similar observation were made by Voller and colleagues who found that this test was positive in 19 out of 20 Tanzanian sera and in all the 41 Iranian patients who were parasitologically positive for malaria. Dutta et al (1982) reported, using P. falciparum antigen, sera at a single dilution (1 : 200) from 143 malaria patients (Group I). 70 patients of varied origin (Group II). 75 random hospital patients (Group III)

and 75 normal healthy subjects (Group IV) were tested at a cut off point equivalent to 95% confidence limit of normal subjects (Group IV), 100.0, 6.6 and 12% cases respectively of Group I to IV gave positive ELISA reaction. Srivastava et al found that using P.falciparum antigen, the ELISA test at 99 percent confidence limit gave 99.3 percent positive results among 143 malaria patients while none of the 70 patients of pyrexia and 75 random hospital patients gave positive reaction. These findings confirm previous reports on other serological tests. (Mathews et al, 1975; Ray et al, 1983; Agarwal et al, 1968 and Wilson et al, 1975). Chandrami et al (1981), Kagan et al (1969), Mahajan et al (1981, 1982) observed lower sensitivity as compared to present study.

The specificity was observed 93.92 percent at cut off titre (7/.40) at which sensitivity and specificity were more acceptable. Similar observation was made by Ray et al (1983) who compared IHA, IIF and ELISA , and did not reveal significant differences as shown by Wilson et al and differences observed in the three tests suggest that antibodies detected may comprise similar and dissimilar classes. Mahajan et al found ELISA to be far superior to IHA and IIF in acute malaria infection using P. knowlesi antigen.

6.2 ELISA Test by age and sex :-

In our study, ELISA value was increased with the increase in age upto the age of about 54 years and the mean ELISA value showed a decreasing trend in the elderly age group i.e. over 55 years at cut off point $\angle$ 40 O.D. (E_{492}) with serum dilution 1 : 400. Our finding corroborate with Voller et al (1980), who observed in a longitudinal study of malaria in West Africa Savanna, in unprotected and protected population after one year of protection that showed ELISA values increased with age and reached a plateau by age 19 - 28 years in unprotected population. In the protected population, the ELISA values were significantly lower in age groups 1-4 to 9-18 years, but there was less difference in the older age groups at cut off point (∇ 0.2) with serum dilution 1 : 100. High ELISA value correlates well with degree of exposure. Malaria control activities result in low ELISA value. ELISA may give negative results in infants with proven parasitaemia (Voller et al. 1980).

In our study, mean ELISA value was higher in males than females. It is due to the fact that males are involved in outdoor activity more frequently than females thus resulted to high degree of malaria exposure. This test has been used in large number of studies of malaria serology (Ambrosio Thomaz et al. 1978; Mårtenson et al. 1979; Srivastava et al. 1981, 1983; Mahajan et al. 1981;

Ray et al. 1983a; Dutta et al. 1982, 1984; Spencer et al. 1979, 1981; Voller et al. 1974 a,b, 1975, 1978, 1980).

Spencer et al (1979 a) used in vitro culture of Pf. falciparum antigen for micro-ELISA. Positive (7 SD) ELISA antibody responses were found in persons with parasitaemia. In all the semi-immune individuals titre were 780, and reciprocal titre rose rapidly to levels 72560 by 2nd to 9th day of patent parasitaemia and gradually decayed after curative therapy. In non-immune individuals titres were lower than in semi-immune individuals. However, positive titres do appear rapidly with patent parasitaemia. In another study (1979 b) they observed discordance between IIF & ELISA in 23% samples from Vietnam and 29.4% from Honduras. ELISA was negative in considerable number of parasitologically positive cases. Adrison et al (1979) observed higher ELISA values in unprotected population in comparison to protected population.

7. Indirect Immunofluorescent Test :

7.1 IIF by Age & Sex :-

Out of 1520 individuals examined for slide positivity and ELISA positivity, 722 individuals were also further analysed by IIF test. All individuals could not be tested due to paucity of time and available resources in this particular study. Comparative conclusion could not be

drawn between IIF & ELISA. However, higher (30.37%) reaction grading was observed in age group 55-64 years, followed by 25-34 age group. The lower (8.11%) reaction grading was observed in age group 10-14 years individuals at 1:32 dilution end point. Similarly, higher (46.22%) positive reaction was observed in age group 45-54 years, followed by 55-64 age groups and lower (24.32%) was observed in 10-14 years age group individuals at 1:64 dilution end point. The reaction grading (2+ and above) for males, was 57.92% and 48.51% at 1:32 dilution and 1:64 dilution respectively. For females, it was 66.78 percent and 52.20 percent at 1:32 dilution and 1:64 dilution end point respectively. In relation to age sero-positivity rate was increasingly higher (9.14%) in age group 25-34 years and lower (1.32%) in age group 1-4 years and thereafter sero-positivity rate showed a decreasing trend upto age group 65+ (1.96%).

Studies carried out in endemic malarious area of Africa, have shown parallelism between the age dependent rise of immunity to malaria and the level of antibodies measured by immunofluorescent techniques (Bray, 1962; McGregor et al. 1965, 1968; Schindler, 1967; Collins, Skinner & Coleman, 1967). With advancing age and increased exposure to degree of transmission, the inhabitants of endemic and hole-endemic areas show a proportion rise of the fluorescent antibody (F.A.) titre. McGregor et al (1965)

indicated that the rate of increase in antibody is rapid in young children but slows down in adolescence and adult life.

Collins et al (1971) in a study at Ethiopia, observed 36.7 percent and 4.3 percent IFA positivity at low and high altitude. This corresponded with other malariometric indices. The positivity was higher among males than females. While studying antibody response in persons previously exposed to malaria, Bruce Chwatt et al (1972) concluded that about 30 percent showed a positive response at low titre against P. falciparum and P. vivax. There was little evidence of persistence of malaria infection in this group.

Warren et al (1975) in a study in Costa Rica observed 0.8 percent and 31.7 percent positivity in population under 15 years and over 15 years respectively. They suggested that positive responses were more likely to be associated with old or imported cases than with current local transmission. Srivastava et al (1983) observed its high diagnostic value since 98 percent of slide positive malaria patients carrying P. falciparum or P. vivax could be diagnosed. Furthermore, positivity observed in patients of pyrexia as well as random hospital patients reflected a low degree of false positivity due to past experience of malaria infection among these cases.

7.2 Validity of IIF Test :-

Out of 722 total ELISA positive individuals examined by IIF, antibodies were detectable in 47.5 percent individuals from study area during transmission season. Some of them were not having malaria at the time of survey. In many of them detectable antibodies might have been due to previous experience to malaria. It was hence considered desirable to find out a cut off titre at which the diagnosis of malaria could be made with reasonable sensitivity and specificity.

The sensitivity and specificity were calculated at cut off titres of 32 and 64. The test was very sensitive at these titres and specificity range from 40.76 percent to 50.0 percent. Whereas, at cut off titre 1:32, though the sensitivity was good (90.00%), the specificity was lower (40.76%). At cut off titre 1:64 though the specificity was slightly better but the sensitivity was poor (77.50%). Taking this into consideration, cut off titre of 32 was taken for study at which sensitivity and specificity both were most acceptable and this was 90.00 percent and 40.76 percent respectively. In previous studies Agarwal et al (1981, 1982), Ray et al (1982, 1983) also found cut off titre of 32 to be most acceptable. Similar specificity was also observed by Ray et al (1983).

Higher specificity 67.9 percent was observed as compared to present study using same antigen (Ray et al. 1982). Whereas sensitivity was much lower in evaluation study conducted by Collins et al (1981) and Warren et al (1975).

Bruce Chwatt et al (1972) concluded that about 50 percent showed a positive response at low titre against P. falciparum and P. vivax.

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CONCLUSIONS

CONCLUSIONS

The study was conducted in rural areas of Jhansi (hypo-endemic for malaria). One thousand five hundred twenty samples were collected during transmission period (September - October 1987). Samples were examined for slide as well as sero-positivity. Also 722 ELISA positive samples, were further analysed for sero-positivity using IIF technique. The observations of the study have led to following conclusions :

1. There was no significant ($P < 0.25$) difference in slide positivity rate (SPR) among different age groups but sero-positivity rate was significantly low ($P < 0.001$) in those aged ≤ 1 - 14 years in comparison to over 15 years age group.

2. No significant ($P > 0.10$, $P > 0.25$) difference was observed in SPR and sero-positivity rate in relation to religion and caste.

3. Significant ($P < 0.01$, $P < 0.001$) difference was observed in both the rates between unmarried individuals and married individuals.

4. Significant ($P > 0.025$, $P < 0.001$) difference was observed in SPR and sero-positivity rate between

illiterates and literates as SPR declined with improvement in literacy status.

5. No significant difference ($P < 0.5$) was observed in SPR for various occupations except individuals classified as other groups. While comparing adults and children, there was significant difference ($P < 0.001$) observed in sero-positivity rate.

6. There was no significant difference ($P > 0.05$) observed in SPR in relation to various social class but significant difference ($P > 0.01$) observed in sero-positivity rate between Social Class II & V.

7. SPR was not significant ($P < 0.25$) in relation with over-crowding but sero-positivity rate was significantly higher ($P < 0.01$) in individuals residing in over-crowded dwellings and keeping cattles within dwellings.

8. Significant difference ($P < 0.001$. $P < 0.001$) was observed in SPR and sero-positivity rate between individuals with hepatomegaly and without hepatomegaly.

9. Significant difference ($P < 0.001$. $P < 0.001$) was observed in SPR and sero-positivity between individuals with splenomegaly and without splenomegaly.

10. SPR and sero-positivity rates were significantly different ($P < 0.10$. $P < 0.001$) between individuals with history of fever and without history of fever.

11. No significant difference ($P > 0.75$) was observed in SPR between individuals with past history of treatment and without past history of treatment but there was significant difference ($P < 0.001$) in sero-positivity rates.

12. Significant difference ($P < 0.001$) was observed in SPR between individuals with high temperature and without temperature.

13. The difference in sero-positivity rates was insignificant among males and females.

14. The sero-positivity rate correlated positively with slide positivity rate.

15. The ELISA values were markedly lower in age groups 1 - 4 to 5 - 14 years, but there was less difference in older age groups. The ELISA values increased with age and reached a plateau by age of 54 years, in protected population.

16. ELISA was found to be highly sensitive (100.0%) and moderately specific (53.92%) test.

17. IIF was found to be sensitive (90.00%) and moderately specific (40.76%) test.

10. Multiple serological tests should be performed for diagnosis of malaria. Rising antibody titre (ELISA) and raised IIF antibody levels alongwith any other positive test, give very strong evidence of malaria, but this needs further evaluation in an area with high incidence of malaria.

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APPENDICES

APPENDIX - I

SERO-EPIDEMIOLOGY OF MALARIA IN RURAL POPULATION

FAMILY SCHEDULE

Date : .....

Village ..... Code No. ..... House No. .....

Name of Head of Family : .....

Religion : Hindu/Muslim/Other(specify)

Caste : Scheduled/Backward/Upper

Main family occupation : .....

Type of family : Joint / Single

Total No.of family members : .....

Total monthly income (average) : Rs. .....

Per capita monthly income : Rs. .....

Social Class : I / II / III / IV / V

No. of living rooms in the family : .....

No. of living units : .....

Over-crowding : Present / Absent

FAMILY COMPOSITION :

Sl. No.	Name	Age	Sex	Relation with Head	Liter-acy status	Marital status	Occu-pation	Income	Name-ths

Signature of Investigator.

APPENDIX - II

SERO-EPIDEMIOLOGY OF MALARIA IN RURAL POPULATION

INDIVIDUAL SCHEDULE

Code No. : .....

Date : .....

Name of individual	:	.....
Age	:	.....
Sex	:	Male / Female
Village & Family No.	:	.....
Sleeping habits	:	Day / Night
Past history of fever	:	.....
• If present, when ?		
• Duration	:	.....
• Temperature	:	.....
• Anaemia	:	Present / Absent
• Hepatomegaly	:	Present / Absent
• Splenomegaly	:	Present / Absent
• Treatment if taken	:	Presumptive only / Presumptive + Radical / None
• Sero-positivity	:	
I.I.F.	:	Present / Absent
ELISA	:	Present / Absent
Period of survey	:	Transmission period / Non-transmission period.

FIELD NOTES IF ANY :

Signature of Investigator.

APPENDIX - III

Carbonate Buffer 9.6 pH :

(a) 8.4 gm $NaHCO_3$ in 100 ml distilled water (Stock Solution-A)

(b) 10.6 gm $Na_2 CO_2$ in 100 ml distilled water (Stock Solution - B).

100 ml of Stock Solution-A + 18.2 ml of Stock Solution-B and dilute to one litre of distilled water.

Phosphate Buffer Solution pH 7.2 :

0.5 M Na_2HPO_4 (35.632 gm/litre) Stock Solution-A

0.5 M $Na\ H_2\ PO_4$ (31.200 gm/litre) Stock Solution-B

A + B dissolved in 200 ml of distilled water (Stock Solution)

40 ml of Stock Solution + 100 ml of 85% Aqueous NaCl.

Dilute to 1 litre with distilled water.

Phosphate Buffer Solution Tween-20 (PBST)

1000 ml PBS with distilled water + 5 ml Tween-20.

Citrate Buffer pH 5.0 :

Stock A 3.84 gm of citric acid in 200 ml.

Stock B 5.68 gm of $Na_2\ HPO_4$ in 200 ml.

35 ml Stock A + 25 ml. Stock B + 50 ml distilled water is equal to 100 ml (Stock Solution).

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